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     (FILE 'USPAT' ENTERED AT 14:23:59 ON 14 MAR 1999)
L1             0 S MLC2 PROMOTER AND ANTISENSE
L2             0 S MLC2 AND PROMOTER AND ANTISENSE
L3             0 S MLC-2 AND PROMOTER AND ANTISENSE
L4          3623 S VECTOR AND ANTISENSE AND EXPRESSION
L5          1119 S L4 AND HEART
L6           230 S L5 AND CARDIAC
L7             4 S L6 AND MLC
L8             4 S L7 AND FY<1996
L9            69 S L4 AND DYSTROPHIN
L10            0 S L9 AND BETA ADRENERGIC
L11           24 S L9 AND RIBOZYME
L12           16 S L11 AND FY<1996
L13           45 S CARDIAC AND MUSCLE AND DYSTROPH? AND ANTISENSE AND FY<19
96
L14           38 S L13 AND (VIRUS OR VIRAL) AND VECTOR
L15           11 S L14 AND RIBOZYME
L16           27 S L14 NOT L15
L17           15 S CARDIAC AND MUSCLE AND BETA ADRENERGIC RECEPTOR AND (ANT
ISE
L18            0 S CARDIAC AND MUSCLE AND NITROGEN MONOXIDE SYNTHASE AND (A
NTI
L19            0 S NITROGEN MONOXIDE SYNTH? AND (ANTISENSE OR RIBOZYME) AND
 FY
L20            0 S ITR AND ONLY TWO AND ADENOVIR? ASSOCIATED AND VECTOR
L21            1 S ITR AND ONLY TWO AND ADENOVIR? AND VECTOR
L22            5 S ITR AND ADENOVIR? AND VECTOR AND CARDIAC AND MUSCLE
L23            0 S 1-5 TI AB
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US PAT NO: 5,792,453 [IMAGE AVAILABLE] L8: 1 of 4
TITLE: Gene transfer-mediated angiogenesis therapy

ABSTRACT:
The transgene-inserted replication-deficit adenovirus **vector** is effectively used in in vivo gene therapy for peripheral vascular disease and **heart** disease, including myocardial ischemia, by a single intra-femoral artery or intracoronary injection directly conducted deeply in the lumen of the one or both femoral or coronary arteries (or graft vessels) in an amount sufficient for transfecting cells in a desired region.

=> d 1-4 ti ab

US PAT NO: 5,792,453 [IMAGE AVAILABLE] L8: 1 of 4
TITLE: Gene transfer-mediated angiogenesis therapy

ABSTRACT:
The transgene-inserted replication-deficit adenovirus **vector** is effectively used in in vivo gene therapy for peripheral vascular disease and **heart** disease, including myocardial ischemia, by a single intra-femoral artery or intracoronary injection directly conducted deeply in the lumen of the one or both femoral or coronary arteries (or graft vessels) in an amount sufficient for transfecting cells in a desired region.

US PAT NO: 5,756,264 [IMAGE AVAILABLE] L8: 2 of 4
TITLE: **Expression vector** systems and method of use

ABSTRACT:
This invention relates to gene therapy by using **vectors** which encode stable MRNA and methods of using such **vectors**. In particular, this invention relates to **vectors** which establish controlled **expression** of recombinant genes within tissues at certain levels. The **vector** includes a 5' flanking region which includes necessary sequences for **expression** of a nucleic acid cassette, a 3' flanking region including a 3' UTR and/or 3' NCR which stabilizes mRNA expressed from the nucleic acid cassette, and a linker which connects the 5' flanking region to a nucleic acid sequence. The linker has a position for inserting a nucleic acid cassette. The linker does not contain the coding sequence of a gene that the linker is naturally associated with. The 3' flanking region is 3' to the position for inserting the nucleic acid cassette. The **expression vectors** of the present invention can also be regulated by a regulatory system and/or constructed with a coating.

US PAT NO: 5,602,102 [IMAGE AVAILABLE] L8: 3 of 4
TITLE: Dipeptidyl peptidase-I inhibitors and uses thereof

ABSTRACT:
Therapeutic agents and methods for the treatment of immunologically mediated diseases and malignancies of myeloid cell or lymphoid cell origin. These particular methods utilize the characterization of particular activation mechanisms important to the progression of these pathologies in humans. Selective inhibition of cell types responsible for precipitating these disorders in humans are provided with therapeutic agents which include peptides capable of inhibiting dipeptidyl

peptidase-I activation of proenzymes present primarily in cytotoxic T-cells and myeloid cells, such as Gly--Phe--CHN.sub.2. **Antisense** oligonucleotides are also characterized which are specific for human dipeptidyl peptidase-I gene which may be used in the treatment of the described disorders.

US PAT NO: 5,429,923 [IMAGE AVAILABLE] L8: 4 of 4
TITLE: Method for detecting hypertrophic cardiomyophathy associated mutations

ABSTRACT:
A method is described for diagnosing individuals as having hypertrophic cardiomyopathy, e.g. familial or sporadic hypertrophic cardiomyopathy. The method provides a useful diagnostic tool which becomes particularly important when testing asymptomatic individuals suspected of having the disease. Symptomatic individuals have a much better chance of being diagnosed properly by a physician. Asymptomatic individuals from families having a history of familial hypertrophic cardiomyopathy may be selectively screened using the method of this invention allowing for a diagnosis prior to the appearance of any symptoms. Individuals having the mutation responsible for the disease may be counseled to take steps which hopefully would prolong their life, i.e. avoid rigorous exercise. The methodology used in the above method also has broad applicability and may be used to detect other disease-associated mutations in DNA obtained from subjects being tested for other disease-associated mutations.

US PAT NO: 5,853,752 [IMAGE AVAILABLE] L16: 1 of 27
TITLE: Methods of preparing gas and gaseous precursor-filled microspheres

ABSTRACT:
Methods of and apparatus for preparing temperature activated gaseous precursor-filled liposomes are described. Gaseous precursor-filled liposomes prepared by these methods are particularly useful, for example, in ultrasonic imaging applications and in therapeutic drug delivery systems.

US PAT NO: 5,849,897 [IMAGE AVAILABLE] L16: 2 of 27
TITLE: Nucleic acid molecule encoding ciliary neurotrophic factor receptor

ABSTRACT:
The present invention relates to the ciliary neurotrophic factor (CNTF) receptor, and provides for CNTF receptor nucleic acid and amino acid sequences. It also relates to (i) assay systems for detecting CNTF activity; (ii) experimental model systems for studying the physiologic role of CNTF; (ii) diagnostic techniques for identifying CNTF-related neurologic conditions; (iv) therapeutic techniques for the treatment of CNTF-related neurologic and muscular conditions, and (v) methods for identifying molecules homologous to CNTF and CNTFR.

US PAT NO: 5,798,224 [IMAGE AVAILABLE] L16: 3 of 27
TITLE: Nucleic acids encoding protocadherin

ABSTRACT:
Polynucleotide sequences encoding novel cadherin-like polypeptides, designated protocadherins, and variants thereof are provided by the invention as well as methods and materials for the recombinant production of the same. Antibody substances specific for protocadherins are also disclosed as useful for modulating the natural binding and/or regulatory activities of the protocadherins.

US PAT NO: 5,795,872 [IMAGE AVAILABLE] L16: 4 of 27
TITLE: DNA construct for immunization

ABSTRACT:
The present invention is directed to a DNA construct which is useful for immunization or gene therapy. The construct of the invention comprises **muscle** specific regulatory elements, such as a promoter or a promoter and one or more enhancer elements, and a DNA sequence under control of the **muscle** specific regulatory elements. Several DNA sequences may be incorporated into the DNA construct. In one embodiment, the DNA sequence codes for an antigen, antigenic determinant or an epitope of an antigen. In a second embodiment, the DNA sequence is a normal **muscle** gene which is effected in a **muscle** disease. In a third embodiment, the DNA sequence is an **antisense** for blocking an abnormal **muscle** gene. In a fourth embodiment, the DNA sequence codes for a protein which circulates in the mammalian blood or lymphatic systems. The present invention is useful for ameliorating the effects of diseases of **muscle** by expression of the normal gene or blocking abnormal gene expression within **muscle** cells, for the heterologous expression of a transgene which codes for a circulating protein or a protein which modifies a disease state in which **muscle** is not primarily involved and for vaccine development.

US PAT NO: 5,770,2[redacted] [IMAGE AVAILABLE] : 5 of 27
TITLE: Therapeutic drug delivery systems

ABSTRACT:
Therapeutic drug delivery systems comprising gas-filled microspheres comprising a therapeutic are described. Methods for employing such microspheres in therapeutic drug delivery applications are also provided. Drug delivery systems comprising gas-filled liposomes having encapsulated therein a drug are preferred. Methods of and apparatus for preparing such liposomes and methods for employing such liposomes in drug delivery applications are also disclosed.

US PAT NO: 5,715,824 [IMAGE AVAILABLE] L16: 6 of 27
TITLE: Methods of preparing gas-filled liposomes

ABSTRACT:
Methods of and apparatus for preparing gas-filled liposomes are described. Gas-filled liposomes prepared by these methods are particularly useful, for example, in ultrasonic imaging applications and in therapeutic drug delivery systems.

US PAT NO: 5,708,143 [IMAGE AVAILABLE] L16: 7 of 27
TITLE: Protocadherin materials and methods

ABSTRACT:
Polynucleotide sequences encoding novel cadherin-like polypeptides, designated protocadherins, and variants thereof are provided by the invention as well as methods and materials for the recombinant production of the same. Antibody substances specific for protocadherins are also disclosed as useful for modulating the natural binding and/or regulatory activities of the protocadherins.

US PAT NO: 5,693,622 [IMAGE AVAILABLE] L16: 8 of 27
TITLE: Expression of exogenous polynucleotide sequences **cardiac muscle** of a mammal

ABSTRACT:
The present invention provides a method for delivering a pharmaceutical polypeptide to the interior of a **cardiac** cell of a vertebrate in vivo, comprising the step of introducing a preparation comprising a pharmaceutically acceptable injectable carrier and naked polynucleotide operatively coding for the polypeptide into the interstitial space of the heart, whereby the naked polynucleotide is taken up into the interior of the cell and has a pharmacological effect on the vertebrate. In a preferred embodiment wherein the polynucleotide encodes polypeptide immunologically foreign to the vertebrate, the delivery method preferably comprises delivering an immunosuppressive agent to the vertebrate to limit immune responses directed to the polypeptide.

US PAT NO: 5,686,073 [IMAGE AVAILABLE] L16: 9 of 27
TITLE: Polyclonal and monoclonal antibodies against a 43 KDA **dystrophin** associated protein

ABSTRACT:
Disclosed are methods for the preparation of polyclonal and monoclonal antibodies which bind specifically to a 43 kDa **dystrophin**-associated. The molecular weight of the 43 kDa protein is determined by electrophoretic separation under denaturing conditions, followed by transfer to a solid support and staining with wheat germ agglutinin. The method includes a step in which the peptide PKNMTPYRSPPPYVP (SEQ ID NO: 15) is administered to stimulate an immune response. Also disclosed are polyclonal and monoclonal antibodies which bind specifically to the 43 kDa **dystrophin**-associated protein.

US PAT NO: 5,683,8[redacted] [IMAGE AVAILABLE] L16: 10 of 27
TITLE: Method [redacted] nucleic acid sequencing

ABSTRACT:
The present invention relates, in general, to a process of enzymatically synthesizing nucleic acids containing nucleotides that are resistant to degradation. The invention further relates to methods of utilizing such nucleic acids in DNA and RNA amplification and sequencing, gene therapy and molecular detection protocols.

US PAT NO: 5,681,735 [IMAGE AVAILABLE] L16: 11 of 27
TITLE: Transcription control element for increasing gene expression in myoblasts

ABSTRACT:

A transcription control element is provided for controlling gene expression in myogenic cells. The transcription control element comprises an isolated DNA segment having an enhancer activity in cultured cells and in non-cultured myogenic cells. The transcription control element is isolated from upstream regions of genes encoding bHLH myogenic regulatory proteins. Specifically, an enhancer element from the upstream region of human myoD and an enhancer element from the upstream region of a quail qmf1 are provided. These myoblast-specific transcription control elements are capable of significantly increasing the levels of gene expression in myogenic cells and are intended to be applied in gene therapy, using myoblast transfer and microinjection techniques, wherein myoblast-specific gene expression is desired or required.

US PAT NO: 5,672,694 [IMAGE AVAILABLE] L16: 12 of 27
TITLE: .beta.-sarcoglycan nucleic acid sequence, and nucleic acid probes

ABSTRACT:
Disclosed herein is a substantially pure nucleic acid sequence encoding a mammalian 43 kDa non-**dystrophin** component (.beta.-sarcoglycan) of the **dystrophin**-glycoprotein complex. Also disclosed are immunogenic peptides which, when used to immunize a mammal, stimulate the production of antibodies which bind specifically to the .beta.-sarcoglycan. Mutations in the .beta.-sarcoglycan gene which are associated with autosomal recessive limb-girdle muscular **dystrophy** are also disclosed. The identification of such mutations enables the design of nucleic acid probes which hybridize specifically to a mutant form of .beta.-sarcoglycan, or the complement thereof, but not to the DNA of the wild-type form of the gene (or the complement thereof), under stringent hybridization conditions. Such probes are useful, for example, in connection with the diagnosis of autosomal recessive limb-girdle muscular **dystrophy**. In addition, the identification of such mutations enables the diagnosis of autosomal recessive limb-girdle muscular **dystrophy** through the use of direct DNA sequencing techniques.

US PAT NO: 5,663,300 [IMAGE AVAILABLE] L16: 13 of 27
TITLE: Protocadherin-42

ABSTRACT:
Polynucleotide sequences encoding novel cadherin-related polypeptides, designated protocadherins, and variants thereof are provided by the invention as well as methods and materials for the recombinant production of the same. Antibody substances specific for protocadherins are also disclosed as useful for modulating the natural binding and/or regulatory activities of the protocadherins.

US PAT NO: 5,648,334 [IMAGE AVAILABLE] L16: 14 of 27
TITLE: Methods of treatment using ciliary neurotrophic factor

ABSTRACT:
The present invention relates to a method of treatment of neuromuscular or **muscle** disorder resulting from the loss of axonal contact with the **muscle** comprising administering an effective amount of ciliary neurotrophic factor. The invention also relates to a method of treatment of a disorder of a type of tissue or cell resulting from the loss of axonal contact with the cell comprising administering an effective amount of ciliary neurotrophic factor in which the type of tissue or cell expresses a CNTF receptor protein.

US PAT NO: 5,643,781 [IMAGE AVAILABLE] L16: 15 of 27
TITLE: DNA encoding protocadherin-42

ABSTRACT:
Polynucleotide sequences encoding novel cadherin-related polypeptides, designated protocadherins, and variants thereof are provided by the invention as well as methods and materials for the recombinant production of the same. Antibody substances specific for protocadherins are also disclosed as useful for modulating the natural binding and/or regulatory activities of the protocadherins.

US PAT NO: 5,591,590 [IMAGE AVAILABLE] L16: 16 of 27
TITLE: Neuronal nicotinic acetylcholine receptor assay

ABSTRACT:
The present invention relates to a family of neuronal nicotinic acetylcholine receptors comprised of neuronal agonist and non-agonist binding subunits, and DNA sequences encoding such subunits. These novel neuronal nicotinic acetylcholine receptor subunits include the agonist binding subunits alpha2, alpha3, alpha4, and alpha5, plus non-agonist binding subunits beta2, beta3 and beta4. Representative cDNA clones that contain the DNA sequences of the invention have been deposited with the American Type Culture Collection for patent purposes.

US PAT NO: 5,585,112 [IMAGE AVAILABLE] L16: 17 of 27
TITLE: Method of preparing gas and gaseous precursor-filled microspheres

ABSTRACT:
Methods of and apparatus for preparing temperature activated gaseous precursor-filled liposomes are described. Gaseous precursor-filled liposomes prepared by these methods are particularly useful, for example, in ultrasonic imaging applications and in therapeutic drug delivery systems.

US PAT NO: 5,580,575 [IMAGE AVAILABLE] L16: 18 of 27
TITLE: Therapeutic drug delivery systems

ABSTRACT:
Therapeutic drug delivery systems comprising gas-filled microspheres comprising a therapeutic are described. Methods for employing such microspheres in therapeutic drug delivery applications are also provided. Drug delivery systems comprising gas-filled liposomes having encapsulated therein a drug are preferred. Methods of and apparatus for preparing such liposomes and methods for employing such liposomes in drug delivery applications are also disclosed.

US PAT NO: 5,542,935 [IMAGE AVAILABLE] L16: 19 of 27
TITLE: Therapeutic delivery systems related applications

ABSTRACT:
Therapeutic delivery systems comprising gaseous precursor-filled microspheres comprising a therapeutic are described. Methods for employing such microspheres in therapeutic delivery applications are also provided. Therapeutic delivery systems comprising gaseous

precursor-filled liposo[redacted] having encapsulated therein a contrast agent or drug are preferred. Methods of and apparatus for prep[redacted]ng such liposomes and methods for employing such liposomes in therapeutic delivery applications are also disclosed.

US PAT NO: 5,538,722 [IMAGE AVAILABLE] L16: 20 of 27
TITLE: Isolation, growth, differentiation and genetic engineering of human **muscle** cells

ABSTRACT:
Myoblasts are produced, conveniently in low or serum-free medium, for use in introduction into a mammalian host, particularly a human host, for treatment of diseases of **muscle** tissue or acting as carriers for genetic capabilities, particularly correcting a genetic defect or for production of a soluble protein, which may serve in a therapy for the mammalian host. Myoblasts introduced into tissue are able to migrate to sites distal from the site of injection, expanding the area of their effect.

US PAT NO: 5,469,854 [IMAGE AVAILABLE] L16: 21 of 27
TITLE: Methods of preparing gas-filled liposomes

ABSTRACT:
Methods of and apparatus for preparing gas-filled liposomes are described. Gas-filled liposomes prepared by these methods are particularly useful, for example, in ultrasonic imaging applications and in therapeutic drug delivery systems.

US PAT NO: 5,466,676 [IMAGE AVAILABLE] L16: 22 of 27
TITLE: Satellite cell proliferation in adult skeletal **muscle**

ABSTRACT:
Novel methods of retroviral-mediated gene transfer for the in vivo corporation and stable expression of eukaryotic or prokaryotic foreign genes in tissues of living animals is described. More specifically, methods of incorporating foreign genes into mitotically active cells are disclosed. The constitutive and stable expression of E. coli .beta.-galactosidase gene under the promoter control of the Moloney murine leukemia **virus** long terminal repeat is employed as a particularly preferred embodiment, by way of example, establishes the model upon which the incorporation of a foreign gene into a mitotically-active living eukaryotic tissue is based.
Use of the described methods in therapeutic treatments for genetic diseases, such as those muscular degenerative diseases, is also presented. In **muscle** tissue, the described processes result in genetically-altered satellite cells which proliferate daughter myoblasts which preferentially fuse to form a single undamaged **muscle** fiber replacing damaged **muscle** tissue in a treated animal. The retroviral **vector**, by way of example, includes a **dystrophin** gene construct for use in treating muscular **dystrophy**.
The present invention also comprises an experimental model utilizable in the study of the physiological regulation of skeletal **muscle** gene expression in intact animals.

US PAT NO: 5,449,616 [IMAGE AVAILABLE] L16: 23 of 27
TITLE: Nucleic acid encoding **dystrophin**-associated protein

ABSTRACT:
Disclosed are nucleic acid sequences encoding components of the **dystrophin**-glycoprotein complex. The components include dystroglycan, the 50 kDa protein component and the 59 kDa protein component. Also disclosed are compositions and methods which relate to the disclosed sequences.

US PAT NO: 5,429,923 [IMAGE AVAILABLE] L16: 24 of 27

TITLE: Method f[redacted] detecting hypertrophic cardiom[redacted]ophathy associ[redacted]d mutations

ABSTRACT:
A method is described for diagnosing individuals as having hypertrophic cardiomyopathy, e.g. familial or sporadic hypertrophic cardiomyopathy. The method provides a useful diagnostic tool which becomes particularly important when testing asymptomatic individuals suspected of having the disease. Symptomatic individuals have a much better chance of being diagnosed properly by a physician. Asymptomatic individuals from families having a history of familial hypertrophic cardiomyopathy may be selectively screened using the method of this invention allowing for a diagnosis prior to the appearance of any symptoms. Individuals having the mutation responsible for the disease may be counseled to take steps which hopefully would prolong their life, i.e. avoid rigorous exercise. The methodology used in the above method also has broad applicability and may be used to detect other disease-associated mutations in DNA obtained from subjects being tested for other disease-associated mutations.

US PAT NO: 5,426,177 [IMAGE AVAILABLE] L16: 25 of 27
TITLE: Ciliary neurotrophic factor receptor

ABSTRACT:
The present invention relates to the ciliary neurotrophic factor (CNTF) receptor, and provides for CNTF receptor nucleic acid and amino acid sequences. It also relates to (i) assay systems for detecting CNTF activity; (ii) experimental model systems for studying the physiologic role of CNTF; (ii) diagnostic techniques for identifying CNTF-related neurologic conditions; (iv) therapeutic techniques for the treatment of CNTF-related neurologic and muscular conditions, and (v) methods for identifying molecules homologous to CNTF and CNTFR.

US PAT NO: 5,371,188 [IMAGE AVAILABLE] L16: 26 of 27
TITLE: Neuronal nicotinic acetylcholine receptor compositions

ABSTRACT:
The present invention relates to a family of neuronal nicotinic acetylcholine receptors comprised of neuronal agonist and non-agonist binding subunits, and DNA sequences encoding such subunits. These novel neuronal nicotinic acetylcholine receptor subunits include the agonist binding subunits alpha2, alpha3, alpha4, and alpha5, plus non-agonist binding subunits beta2, beta3 and beta4. Representative cDNA clones that contain the DNA sequences of the invention have been deposited with the American Type Culture Collection for patent purposes.

US PAT NO: 5,298,422 [IMAGE AVAILABLE] L16: 27 of 27
TITLE: Myogenic **vector** systems

ABSTRACT:
The present invention includes a Myogenic **vector** system (MVS) for the expression of a nucleic acid sequence in myogenic tissue. The MVS contains a promoter, a cassette with the sequence of interest, a 3' untranslated region (3' UTR) and contiguous noncoding region (NCR). Further enhancements can include the addition of a leader sequence, intron sequence, initiation codon and specific

This invention was partially supported by a grant from the United States government under HL-38401 awarded by the National Institute of Health. The Government has certain rights in the invention.

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=> s cardiac and muscle and beta adrenergic receptor and (antisense or
ribozyme) and fy<1996

         19922 CARDIAC
            33 CARDIACS
         19947 CARDIAC
                 (CARDIAC OR CARDIACS)
         32446 MUSCLE
         14984 MUSCLES
         39591 MUSCLE
                 (MUSCLE OR MUSCLES)
        180597 BETA
           410 BETAS
        180676 BETA
                 (BETA OR BETAS)
          4137 ADRENERGIC
            98 ADRENERGICS
          4194 ADRENERGIC
                 (ADRENERGIC OR ADRENERGICS)
         31647 RECEPTOR
         21043 RECEPTORS
         37491 RECEPTOR
                 (RECEPTOR OR RECEPTORS)
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                 (BETA(W)ADRENERGIC(W)RECEPTOR)
          4693 ANTISENSE
             1 ANTISENSES
          4693 ANTISENSE
                 (ANTISENSE OR ANTISENSES)
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       2237842 FY<1996
L17          15 CARDIAC AND MUSCLE AND BETA ADRENERGIC RECEPTOR AND (ANTISE
NSE
                OR RIBOZYME) AND FY<1996

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US PAT NO:      5,858,684 [IMAGE AVAILABLE]            L17: 1 of 15
TITLE:          Method of screening calcium receptor-active molecules

ABSTRACT:
The present invention relates to the different roles inorganic ion
receptors have in cellular and body processes. The present invention
features: (1) molecules which can modulate one or more inorganic ion
receptor activities, preferably the molecule can mimic or block an effect
of an extracellular ion on a cell having an inorganic ion receptor, more
preferably the extracellular ion is Ca.sup.2+  and the effect is on a
cell having a calcium receptor; (2) inorganic ion receptor proteins and
fragments thereof, preferably calcium receptor proteins and fragments
thereof; (3) nucleic acids encoding inorganic ion receptor proteins and
fragments thereof, preferably calcium receptor proteins and fragments
thereof; (4) antibodies and fragments thereof, targeted to inorganic ion
receptor proteins, preferably calcium receptor protein; and (5) uses of
such molecules, proteins, nucleic acids and antibodies.
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US PAT NO: 5,844,0[redacted] [IMAGE AVAILABLE] : 2 of 15
TITLE: Cytostatin I

ABSTRACT:
A human cytostatin I polypeptide and DNA encoding such polypeptide and a procedure for producing such polypeptide by recombinant techniques is disclosed. Also disclosed are methods for utilizing such polypeptide for the treatment of cancers, particularly breast cancer, leukemias, and other metastases.

US PAT NO: 5,830,848 [IMAGE AVAILABLE] L17: 3 of 15
TITLE: Method and agents for inducement of endogenous nitric oxide synthase for control and management of labor during pregnancy

ABSTRACT:
A method and agents for endogenous control, treatment, management and prevention of preterm labor by inducement of endogenous nitric oxide synthase. The method for endogenous production of nitric oxide in myometrium involves administering to a pregnant mammal a cytokine, hormone or growth factor agent able to induce production of nitric oxide or nitric oxide synthase. A non-invasive diagnostic procedure for detecting the presence and/or impending onset of premature labor.

US PAT NO: 5,817,477 [IMAGE AVAILABLE] L17: 4 of 15
TITLE: Adrenergic receptor

ABSTRACT:
A human adrenergic receptor polypeptide and DNA (RNA) encoding such polypeptide and a procedure for producing such polypeptide by recombinant techniques is disclosed. Also disclosed are agonists for the adrenergic receptor polypeptide which may be used therapeutically to stimulate the adrenergic receptor and antagonist inhibitors against such adrenergic receptor polypeptides and their use therapeutically to antagonize the adrenergic receptor. Also disclosed are diagnostic methods for detecting mutations in the polynucleotides of the present invention and for detecting levels of the soluble polypeptides in samples derived from a host.

US PAT NO: 5,792,453 [IMAGE AVAILABLE] L17: 5 of 15
TITLE: Gene transfer-mediated angiogenesis therapy

ABSTRACT:
The transgene-inserted replication-deficit adenovirus vector is effectively used in in vivo gene therapy for peripheral vascular disease and heart disease, including myocardial ischemia, by a single intra-femoral artery or intracoronary injection directly conducted deeply in the lumen of the one or both femoral or coronary arteries (or graft vessels) in an amount sufficient for transfecting cells in a desired region.

US PAT NO: 5,766,879 [IMAGE AVAILABLE] L17: 6 of 15
TITLE: DNA encoding 5-HT.sub.4 serotonin receptors and uses thereof

ABSTRACT:

This invention provides an isolated nucleic acid molecule encoding a mammalian 5-HT.sub.4 receptor and an isolated nucleic acid molecule encoding a human 5-HT.sub.4 receptor, an isolated protein which is a mammalian 5-HT.sub.4 receptor, an isolated protein which is a human 5-HT.sub.4 receptor, vectors comprising an isolated nucleic acid molecule encoding a mammalian 5-HT.sub.4 receptor, vectors comprising an isolated nucleic acid molecule encoding a human 5-HT.sub.4 receptor, mammalian

cells comprising such [redacted]tors, antibodies directed to t[redacted] 5-HT.sub.4 receptor, nucleic acid [redacted]robes useful for detecting nucl[redacted]c acid encoding a mammalian or human 5-HT.sub.4 receptor, **antisense** oligonucleotides complementary to any sequences of a nucleic acid molecule which encodes a mammalian or human 5-HT.sub.4 receptor, pharmaceutical compounds related to the human 5-HT.sub.4 receptor, and nonhuman transgenic animals which express DNA encoding a normal or a mutant mammalian or human 5-HT.sub.4 receptor. This invention further provides methods for determining ligand binding, detecting expression, drug screening, and treatments for alleviating abnormalities associated with a human 5-HT.sub.4 receptor.

US PAT NO: 5,763,569 [IMAGE AVAILABLE] L17: 7 of 15
TITLE: Calcium receptor-active molecules

ABSTRACT:
The present invention features calcium receptor polypeptides and fragments thereof. Uses of a calcium receptor polypeptide include providing a polypeptide having the activity of a calcium receptor polypeptide. Calcium receptor polypeptide fragments can be used, for example, to generate antibodies to a calcium receptor polypeptide.

US PAT NO: 5,733,728 [IMAGE AVAILABLE] L17: 8 of 15
TITLE: Detecting and treating heart failure

ABSTRACT:
Methods are disclosed for detecting and treating heart failure which are based on the down-regulatory activity of the cytoplasmic RNA-binding polypeptide, AUF1 (A+U-rich element RNA-binding/degradation Factor) toward **.beta.-adrenergic receptors**. Methods are disclosed for detecting pharmacologicals that inhibit the down-regulatory activity of AUF1 polypeptide for .beta.-adrenergic activity. Methods are disclosed for treating a patient with a high level of the AUF1 gene.

US PAT NO: 5,688,938 [IMAGE AVAILABLE] L17: 9 of 15
TITLE: Calcium receptor-active molecules

ABSTRACT:
The present invention relates to the different roles inorganic ion receptors have in cellular and body processes. The present invention features: (1) molecules which can modulate one or more inorganic ion receptor activities, preferably the molecule can mimic or block an effect of an extracellular ion on a cell having an inorganic ion receptor, more preferably the extracellular ion is Ca.sup.2+ and the effect is on a cell having a calcium receptor; (2) inorganic ion receptor proteins and fragments thereof, preferably calcium receptor proteins and fragments thereof; (3) nucleic acids encoding inorganic ion receptor proteins and fragments thereof, preferably calcium receptor proteins and fragments thereof; (4) antibodies and fragments thereof, targeted to inorganic ion receptor proteins, preferably calcium receptor protein; and (5) uses of such molecules, proteins, nucleic acids and antibodies.

US PAT NO: 5,658,758 [IMAGE AVAILABLE] L17: 10 of 15
TITLE: Polynucleotides encoding cytostatin I

ABSTRACT:
A human cytostatin I polypeptide and DNA encoding such polypeptide and a procedure for producing such polypeptide by recombinant techniques is disclosed. Also disclosed are methods for utilizing such polypeptide for the treatment of cancers, particularly breast cancer, leukemias, and other matastases.

US PAT NO: 5,639,652 [IMAGE AVAILABLE] L17: 11 of 15
TITLE: DNA encoding a human 5-HT.sub.1F receptor and uses thereof

ABSTRACT:

This invention provides an isolated nucleic acid molecule encoding a human 5-HT.sub.1F receptor, an isolated protein which is a human 5-HT.sub.1F receptor, vectors comprising an isolated nucleic acid molecule encoding a human 5-HT.sub.1F receptors, mammalian cells comprising such vectors, antibodies directed to the human 5-HT.sub.1F receptor, nucleic acid probes useful for detecting nucleic acid encoding human 5-HT.sub.1F receptors, **antisense** oligonucleotides complementary to any sequences of a nucleic acid molecule which encodes a human 5-HT.sub.1F receptor, pharmaceutical compounds related to human 5-HT.sub.1F receptors, and nonhuman transgenic animals which express DNA a normal or a mutant human 5-HT.sub.1F receptor. This invention further provides methods for determining ligand binding, detecting expression, drug screening, and treatment involving the human 5-HT.sub.1F receptor.

US PAT NO: 5,621,079 [IMAGE AVAILABLE] L17: 12 of 15
TITLE: Neuropeptide Y receptor

ABSTRACT:
A novel mammalian neuropeptide Y receptor and method of making the receptor are provided. The invention includes DNA encoding the receptor, the receptor, assays employing the receptor, cells expressing the receptor, antibodies which bind specifically to the receptor, RNA encoded by the DNA sequence or its complementary sequence, and single-stranded DNA with a sequence complementary to the RNA which encodes the receptor. The receptor and assays employing the receptor are useful for identifying compounds which bind to the receptor, including specific modulators of the receptor. Such compounds are useful for treating a variety of disease conditions, including obesity, diabetes, anxiety, hypertension, cocaine withdrawal, congestive heart failure, memory enhancement, **cardiac** and cerebral vasospasm, pheochromocytoma and ganglioneuroblastoma, and Huntington's, Alzheimer's and Parkinson's diseases.

US PAT NO: 5,599,673 [IMAGE AVAILABLE] L17: 13 of 15
TITLE: Long QT syndrome genes

ABSTRACT:
The invention relates to the identification of the molecular basis of long QT syndrome. More specifically, the invention has identified that SCN5A and HERG cause long QT syndrome. Molecular variants of the SCN5A and HERG genes contribute to the syndrome. The analysis of these genes will provide an early diagnosis of subjects with long QT syndrome. The diagnostic methods comprise analyzing the nucleic acid sequences of the SCN5A or HERG genes of an individual to be tested and comparing them with the nucleic acid sequence of the native, nonvariant genes. Alternatively, the amino acid sequences of SCN5A or HERG may be analyzed for mutations which cause long QT syndrome. Presymptomatic diagnosis of long QT syndrome will enable practitioners to treat this disorder using existing medical therapy.

US PAT NO: 5,525,621 [IMAGE AVAILABLE] L17: 14 of 15
TITLE: Imidazole derivatives as protective agents in reperfusion injury and severe inflammatory responses

ABSTRACT:
Compounds of the formula ##STR1## wherein the substituents are as defined in the disclosure. The compounds are particularly useful for inhibiting damage to a variety of mammalian tissue that are jeopardized, for example, during runaway inflammatory conditions due to the damaging presence of singlet oxygen, the hydroxyl radical, cytokines and growth factors. The compounds are also useful in inhibiting damage to **cardiac** and central nervous system tissues during reperfusion.

US PAT NO: 5,472,866 [IMAGE AVAILABLE] L17: 15 of 15
TITLE: DNA encoding 5-HT.sub.4A serotonin receptors

ABSTRACT:
This invention provides an isolated nucleic acid molecule encoding a mammalian 5-HT.sub.4A receptor and an isolated nucleic acid molecule encoding a human 5-HT.sub.4A receptor, an isolated protein which is a mammalian 5-HT.sub.4A receptor, an isolated protein which is a human 5-HT.sub.4A receptor, vectors comprising an isolated nucleic acid molecule encoding a mammalian 5-HT.sub.4A receptor, vectors comprising an isolated nucleic acid molecule encoding a human 5-HT.sub.4A receptor, mammalian cells comprising such vectors, antibodies directed to the 5-HT.sub.4A receptor, nucleic acid probes useful for detecting nucleic acid encoding a mammalian or human 5-HT.sub.4A receptor, **antisense** oligonucleotides complementary to any sequences of a nucleic acid molecule which encodes a mammalian or human 5-HT.sub.4A receptor, pharmaceutical compounds related to the human 5-HT.sub.4A receptor, and nonhuman transgenic animals which express DNA encoding a normal or a mutant mammalian or human 5-HT.sub.4A receptor. This invention further provides methods for determining ligand binding, detecting expression, drug screening, and treatments for alleviating abnormalities associated with a human 5-HT.sub.4A receptor.

US PAT NO: 5,801,030 [IMAGE AVAILABLE] L21: 1 of 1
TITLE: Methods and **vectors** for site-specific recombination

ABSTRACT:
The present invention provides methods for site-specific recombination in a cell, as well as **vectors** which can be employed in such methods. The methods and **vectors** of the present invention can be used to obtain persistent gene expression in a cell and to modulate gene expression. One preferred method according to the invention comprises contacting a cell with a **vector** comprising an origin of replication functional in mammalian cells located between first and second recombining sites located in parallel. Another preferred method comprises, in part, contacting a cell with a **vector** comprising first and second recombining sites in antiparallel orientations such that the **vector** is internalized by the cell. In both methods, the cell is further provided with a site-specific recombinase that effects recombination between the first and second recombining sites of the **vector**.

US PAT NO: 5,866,552 [IMAGE AVAILABLE] L22: 1 of 5
TITLE: Method for expressing a gene in the absence of an immune response

ABSTRACT:
A method of gene transfer involving administering a recombinant adeno-associated virus (AAV) bearing the desired gene into the **muscle** of the animal is described.

US PAT NO: 5,858,351 [IMAGE AVAILABLE] L22: 2 of 5
TITLE: Methods for delivering DNA to **muscle** cells using recombinant adeno-associated virus **vectors**

ABSTRACT:
The use of recombinant adeno-associated virus (AAV) virions for delivery of DNA molecules to **muscle** cells and tissue is disclosed. The invention allows for the direct, in vivo injection of recombinant AAV virions into **muscle** tissue, e.g., by intramuscular injection, as well as for the in vitro transduction of **muscle** cells which can subsequently be introduced into a subject for treatment. The invention provides for sustained, high-level expression of the delivered gene and for in vivo secretion of the therapeutic protein from transduced **muscle** cells such that systemic delivery is achieved.

US PAT NO: 5,851,521 [IMAGE AVAILABLE] L22: 3 of 5
TITLE: Viral **vectors** and their use for treating hyperproliferative disorders, in particular restenosis

ABSTRACT:
The present invention relates to replication defective recombinant viruses which contain at least one inserted gene encoding all or part of the protein GAX or of a variant of this protein, and to their therapeutic use, in particular for treating post-angioplastic restenosis.

US PAT NO: 5,846,528 [IMAGE AVAILABLE] L22: 4 of 5
TITLE: Treating anemia using recombinant adeno-associated virus virions comprising an EPO DNA sequence

ABSTRACT:
The use of recombinant adeno-associated virus (AAV) virions for delivery of DNA molecules to **muscle** cells and tissue in the treatment of anemia is disclosed. The invention allows for the direct, in vivo injection of recombinant AAV virions into **muscle** tissue, e.g., by intramuscular injection, as well as for the in vitro transduction of **muscle** cells which can subsequently be introduced into a subject for treatment. The invention provides for sustained, high-level expression of a delivered nucleotide sequence encoding erythropoietin, and for in vivo secretion thereof from transduced **muscle** cells such that systemic delivery is achieved.

US PAT NO: 5,830,461 [IMAGE AVAILABLE] L22: 5 of 5
TITLE: Methods for promoting wound healing and treating transplant-associated vasculopathy

ABSTRACT:
The present invention provides a method of promoting the closure of a wound in a patient. This method involves transferring exogenous iNOS to

the region of the wou[redacted] whereby a product of iNOS is p[redacted]uced in the region of the wound t[redacted]promote the closure of the woun[redacted]

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show files
File 155:MEDLINE(R) 1966-1999/Mar W4
         (c) format only 1999 Dialog Corporation
File   5:BIOSIS PREVIEWS(R) 1969-1999/Jan W4
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         (c) 1999 Derwent Publ Ltd
File 315:ChemEng & Biotec Abs 1970-1999/Feb
         (c)1999 RoySocChm,DECHEMA,FizChemie
File 351:DERWENT WPI 1963-1998/UD=9906;UP=9906;UM=9906
         (c)1999 Derwent Info Ltd
File 358:Current BioTech Abs 1983-1999/Feb
          Royal Soc Chem & DECHEMA
?ds

Set     Items   Description
S1      11292   (MYOSIN()LIGHT()CHAIN?) OR MLC2 OR (MLC()2)
S2       2137   S1 AND (REGULATOR? OR (TRANSCRI? AND START?) OR PROMOTER? -
                ?)
S3        292   S2 AND (RECOMBINAN? OR VECTOR? ? OR PLASMID? ? OR CHIMER? -
               OR FUSION?)
S4        756   (MYOSIN()LIGHT()CHAIN()2) OR MLC2 OR (MLC()2)
S5        279   S4 AND (REGULATOR? OR (TRANSCRI? AND START?) OR PROMOTER? -
               ?)
S6         71   S5 AND (RECOMBINAN? OR VECTOR? ? OR PLASMID? ? OR CHIMER? -
               OR FUSION?)
S7         33   RD S6 (unique items)
?t 7/7/all

 7/7/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 1999 Dialog Corporation. All rts. reserv.

09694834   98412461
 Heart-specific  targeting of beta-galactosidase by the ventricle-specific
cardiac  myosin  light  chain  2  promoter using adenovirus  vectors.

  Griscelli  F;  Gilardi-Hebenstreit  P;  Hanania  N;  Franz  WM; Opolon P;
Perricaudet M; Ragot T
  CNRS UMR 1582, Institut Gustave Roussy, Villejuif, France.
  Hum  Gene  Ther (UNITED  STATES)  Sep  1  1998,  9  (13) p1919-28,  ISSN
1043-0342   Journal Code: A12
  Languages: ENGLISH
  Document type: JOURNAL ARTICLE
  Adenoviruses  are  attractive  vectors  for  gene transfer into cardiac
muscle.  However,  their  promiscuous  tissue  tropism,  which  leads to an
ectopic  expression  of  the  transgene,  is  a considerable limitation. To
restrict expression to cardiomyocytes, we have constructed two recombinant
 adenoviruses  (Ad- MLC2 -250betagal and Ad-MLC2 -2100betagal) containing
the  beta-galactosidase  reporter  gene  under  the  control of the 250- or
2100-bp  rat  ventricle-specific  cardiac  myosin  light chain-2v promoter
```

(MLC-2v). Our in vitro and in vivo data have evidenced that the 2100-bp promoter allows stronger beta-galactosidase activity than the 250-bp promoter and that the deleted promoter allows a weak beta-galactosidase expression in skeletal muscle-derived cells in vitro. In contrast to the in vitro results, the highly deleted MLC-2v promoter of 250 pb conserved its heart specificity in in ovo and in vivo when introduced into the adenovirus genome, indicating that the specificity of this promoter is neither altered by the inverted terminal repeat nor by the enhancer of the Ela promoter , both of which located in the 5' flanking region of the promoter . Systemic injections of both recombinant adenoviruses into chicken embryos showed beta-galactosidase expression mainly in the right ventricle of the heart. We have confirmed the cardiac specificity of both promoters in mammalian species after injection of both recombinant adenoviruses into the heart of adult rats in vivo. The comparison of both promoters in vitro and in vivo has shown that the 250-bp MLC-2v promoter is 80% less active than the 2100-bp MLC-2v promoter and has enabled us to conclude that the MLC-2v promoter of 2100 bp is the most appropriate for efficient expression of a reporter gene or a therapeutic cardiac gene (e.g., SERCA2a or minidystrophin gene).

7/7/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09410517 98120353
Use of DNA injection for identification of slow nerve-dependent regions of the MLC2s gene.
Lupa-Kimball VA; Esser KA
School of Kinesiology, University of Illinois, Chicago 60608, USA.
Am J Physiol (UNITED STATES) Jan 1998, 274 (1 Pt 1) pC229-35, ISSN 0002-9513 Journal Code: 3U8
Contract/Grant No.: AR-43349, AR, NIAMS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
It has been well established that expression of slow contractile protein genes in skeletal muscle is regulated, in part, by activity from slow motoneurons. However, very little is understood about the mechanism by which neural activity regulates transcription of slow isoform genes. The purpose of this investigation was first to more fully define the in vivo DNA injection technique for use in both fast-twitch and slow-twitch muscles and second to use the injection technique for the identification of slow nerve-dependent regions of the myosin light chain 2 slow (MLC2s) gene. Initial experiments determined that the same amount of plasmid DNA was taken up by both the slow-twitch soleus and fast-twitch extensor digitorum longus (EDL) muscles and that injection of from 0.5 to 10 micrograms DNA/muscle is ideal for analysis of promoter activity during regeneration. This technique was subsequently used to identify that the region from -800 to +12 base pairs of MLC2s gene directed approximately 100 times higher activity in the innervated soleus than in innervated EDL, denervated soleus, or denervated EDL muscles. Placing the introns upstream of either the MLC2s or SV40 promoter increased expression 5- and 2.7-fold, respectively, in innervated soleus but not in innervated EDL,

denervated soleus, or denervated EDL muscles. These results demonstrate that 1) in vivo DNA injection is a sensitive assay for promoter analysis in both fast-twitch and slow-twitch skeletal muscles and 2) both 5' flanking and intronic regions of the MLC2s gene can independently and synergistically direct slow nerve-dependent transcription in vivo.

7/7/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09377520 98077010

Analysis of tissue-specific gene delivery by recombinant adenoviruses containing cardiac-specific promoters.

Franz WM; Rothmann T; Frey N; Katus HA

Medizinische Klinik II, Medizinische Universitat zu Lubeck, Germany. franz@medinf.mu-luebeck.de

Cardiovasc Res (NETHERLANDS) Sep 1997, 35 (3) p560-6, ISSN 0008-6363 Journal Code: COR

Languages: ENGLISH

Document type: JOURNAL ARTICLE

OBJECTIVE: To approach heart muscle diseases by gene transfer, an adenoviral vector system was intended to be established suitable for gene expression in ventricular and/or atrial myocardium. METHODS: Two adenoviral vectors (Ad-mhcLuc, Ad-mlcLuc) were constructed, in which the luciferase reporter gene is under control of either the ventricle-specific myosin light chain - 2 (mlc-2v) or the atrial- and ventricular-specific alpha-myosin heavy chain (alpha-mhc) promoter . For controls, a recombinant adenovirus without promoter (Ad-Luc) and one with the Rous sarcoma virus (rsv) promoter (Ad-rsvLuc) were generated. A volume of 20 microliters containing 2 x 10(9) plaque forming units (pfu) of the recombinant adenoviruses Ad-mhcLuc, Ad-mlcLuc, Ad-rsvLuc or Ad-Luc was injected into the cardiac cavity or the quadriceps femoris muscle of neonatal rats. After five days animals were sacrificed and nine different tissues were analyzed for reporter gene expression by detection of light activity relative to mg of tissue. RESULTS: Injections of recombinant adenoviruses into the cardiac cavity of neonatal rats resulted in heart-specific gene expression of Ad-mlcLuc (20 fold of Ad-Luc; 11% of Ad-rsvLuc), whereas Ad-mhcLuc gave mainly luciferase activity in the heart (6.5-fold of Ad-Luc; 3% of Ad-rsvLuc) with additional activity in lung and liver (2-4 fold of Ad-Luc). In the ventricular tissue Ad-mlcLuc revealed a 35-fold higher luciferase activity, whereas Ad-mhcLuc, Ad-rsvLuc and Ad-Luc showed only 2-fold higher luciferase activities compared to the atrium. Viral DNA in atrial and ventricular tissue was detected by PCR at approximately the same abundance independent of the injected type of adenovirus. Direct injection of Ad-mhcLuc and Ad-mlcLuc into the thigh muscle revealed only background luciferase activities. CONCLUSIONS: In the adenoviral system only the mlc-2v promoter may fulfil the safety requirements for a myocardial specific gene expression with a high selectivity for the ventricular myocardium, thus providing a promising tool for future gene therapy of cardiomyopathies.

7/7/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09222324 96157885
Inhibition of a signaling pathway in cardiac muscle cells by active mitogen-activated protein kinase kinase.
Thorburn J; Carlson M; Mansour SJ; Chien KR; Ahn NG; Thorburn A
Program in Human Molecular Biology and Genetics, Eccles Institute of Human Genetics, University of Utah, Salt Lake City 84112, USA.
Mol Biol Cell (UNITED STATES) Nov 1995, 6 (11) p1479-90, ISSN 1059-1524 Journal Code: BAU
Contract/Grant No.: HL-52010, HL, NHLBI; GM-48521, GM, NIGMS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Signaling via the Ras pathway involves sequential activation of Ras, Raf-1, mitogen-activated protein kinase kinase (MKK), and the extracellular signal-regulated (ERK) group of mitogen-activated protein (MAP) kinases. Expression from the c-Fos, atrial natriuretic factor (ANF), and myosin light chain -2 (MLC -2) promoters during phenylephrine-induced cardiac muscle cell hypertrophy requires activation of this pathway. Furthermore, constitutively active Ras or Raf-1 can mimic the action of phenylephrine in inducing expression from these promoters . In this study, we tested whether constitutively active MKK, the molecule immediately downstream of Raf, was sufficient to induce expression. Expression of constitutively active MKK induce ERK2 kinase activity and caused expression from the c-Fos promoter , but did not significantly activate expression of reporter genes under the control of either the ANF or MLC -2 promoters . Expression of CL100, a phosphatase that inactivates ERKs, prevented expression from all of the promoters . Taken together, these data suggest that ERK activation is required for expression from the Fos, ANF, and MLC - 2 promoters but MKK and ERK activation is sufficient for expression only from the Fos promoter . Constitutively active MKK synergized with phenylephrine to increase expression from a c-Fos- or an AP1-driven reporter. However, active MKK inhibited phenylephrine- and Raf-1-induced expression from the ANF and MLC -2 promoters . A DNA sequence in the MLC -2 promoter that is a target for inhibition by active MKK, but not CL100, was mapped to a previously characterized DNA element (HF1) that is responsible for cardiac specificity. Thus, activation of cardiac gene expression during phenylephrine-induced hypertrophy requires ERK activation but constitutive activation by MKK can inhibit expression by targeting a DNA element that controls the cardiac specificity of gene expression.

7/7/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09123221 97364047
Retinoic acid accelerates embryonic stem cell-derived cardiac differentiation and enhances development of ventricular cardiomyocytes.
Wobus AM; Kaomei G; Shan J; Wellner MC; Rohwedel J; Ji Guanju; Fleischmann B; Katus HA; Hescheler J; Franz WM

Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany.

J Mol Cell Cardiol (ENGLAND) Jun 1997, 29 (6) p1525-39, ISSN 0022-2828 Journal Code: J72

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pluripotent embryonic stem (ES) cells spontaneously differentiate via embryo-like aggregates into cardiomyocytes of pacemaker-, atrium- and ventricle-like type, which can be distinguished by their specific patterns of action potentials. It has been shown that retinoic acid (RA) treatment during ES cell differentiation increases the number of cardiomyocytes in a time- and concentration-dependent manner. In order to test the effect of RA on cardiomyocyte differentiation and specialization into ventricle-like cardiomyocytes, we studied gene expression of beta-galactosidase driven by the ventricular myosin light chain -2 (MLC-2v) promoter as an indicator for ventricular differentiation. Clones containing the stably integrated expression vector pGNA/MLC - 2 .1 were selected, which revealed an increase of beta-galactosidase activity in cardiomyocytes of embryoid bodies at day 7 + 16. RA, both, in the all-trans and in the 9-cis configuration resulted in a significant acceleration of cardiomyocyte differentiation and a transient increase of beta-galactosidase activity. To test whether this acceleration of cardiac differentiation and RA-induced increase of the MLC-2v promotor/beta-galactosidase activity reflects an increase of cardiac- and ventricle-specific gene expression, a semi-quantitative RT-PCR analysis was performed for alpha-cardiac myosin heavy chain (alpha-MHC) and MLC-2v genes. It was shown that both 10(-8) M and 10(-9) M RA resulted in an increased level of alpha-cardiac MHC and MLC-2v mRNA in embryoid bodies in early, but not in terminal developmental stages. This led us to the conclusion that the RA-induced accelerated expression of cardiac-specific genes results in an enhanced development of ventricular cardiomyocytes. An increased number of ventricle-like cells after RA treatment was also found by patch-clamp analysis. The number of cardiomyocytes with Purkinje- and ventricle-like properties was shown to be increased by RA, whereas the number of pacemaker- and atrium-like cells was reduced and early pacemaker cells were not quantitatively affected.

7/7/6 (Item 6 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

09114681 97364784

Two distinct factor-binding DNA elements in cardiac myosin light chain 2 gene are essential for repression of its expression in skeletal muscle. Isolation of a cDNA clone for repressor protein Nished.

Dhar M; Mascareno EM; Siddiqui MA

Center for Cardiovascular and Muscle Research, Department of Anatomy and Cell Biology, State University of New York, Brooklyn, New York 11203, USA.

J Biol Chem (UNITED STATES) Jul 18 1997, 272 (29) p18490-7, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: AR41923, AR, NIAMS

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The expression of the cardiac myosin light chain 2 (MLC2) gene is repressed in skeletal muscle as a result of the negative regulation of its transcription. Two regulatory elements, the cardiac specific sequence (CSS) located upstream (-360 base pairs) and a downstream negative modulatory sequence (NMS), which function in concert with each other, are required for repression of the MLC2 promoter activity in skeletal muscle. Individually, CSS and NMS have no effect. Transient transfection analysis with recombinant plasmids indicated that CSS- and NMS-mediated repression of transcription is position- and orientation-dependent and is transferable to heterologous promoters . A minimal conserved motif, GAAG/CTTC, present in both CSS and NMS, is responsible for repression as the mutation in the core CTTC sequence alone was sufficient to abrogate its repressor activity. The DNA binding assay by gel mobility shift analysis revealed that one of the two complexes, CSSBP2, is significantly enriched in embryonic skeletal muscle relative to cardiac muscle. In extracts from adult skeletal muscle, where the cardiac MLC2 expression is suppressed, both complexes, CSSBP1 and CSSBP2, were present, whereas the cardiac muscle extracts contained CSSBP1 alone, suggesting that the protein(s) in the CSSBP2 complex accounts for the negative regulation of cardiac MLC2 in skeletal muscle. A partial cDNA clone (Nished) specific for the candidate repressor factor was isolated by expression screening of the skeletal muscle cDNA library by multimerized CSS-DNA as probe. The recombinant Nished protein binds to the CSS-DNA, but not to DeltaCSS-DNA where the core CTTC sequence was mutated. The amino acid sequence of Nished showed a significant structural similarity to the sequence of transcription factor "runt," a known repressor of gap and pair-rule gene expression in Drosophila.

7/7/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09055831 97325049

Expression from cardiomyocyte-specific promoter after adenovirus-mediated gene transfer in vitro and in vivo.

Griscelli F; Opolon P; Chianale C; Di Falco N; Franz WM; Perricaudet M; Ragot T

Laboratoire de genetique des virus oncogenes, CNRS Ura 1301, Villejuif, France.

C R Acad Sci III (FRANCE) Feb 1997, 320 (2) p103-12, ISSN 0764-4469 Journal Code: CA1

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Adenoviruses are very attractive vectors for gene transfer into the cardiac muscle; however, their promiscuous tissue tropism, leading to an ectopic expression of the transgene, is a considerable practical limitation. To restrict expression of a reporter gene in cultured cardiomyocytes and in the heart of the rat, we have constructed a recombinant adenovirus (Ad- MLC2 beta gal) containing the beta-galactosidase gene under the control of the rat ventricle-specific cardiac myosin light chain 2 (MLC-2v) promoter . We show in this work that the MLC-2v promoter inside the adenoviral genome retains its

cardiac specificity in vitro in cultured cardiomyocytes as well as in vivo in the animal heart. Northern blot studies after Ad-MLC2 beta gal infection show significant transcription only in cells derived from the cardiac muscle and not from the skeletal muscle. Quantitative analysis of the beta-galactosidase activity in a number of cell lines also confirms this result. The level of beta-galactosidase expression in rat neonatal cardiomyocytes infected with Ad-MLC2 beta gal is 8% of that found when primary cells are infected with Ad-RSV beta gal (containing a beta-galactosidase gene under the control of the Rous sarcoma virus promoter). The cardiomyocytes-specific expression is also found after injection of Ad-MLC2 beta gal directly into the rat myocardium, although the viral genome can be detected by polymerase chain reaction (PCR) in other tissues. Lack of expression after direct injection into liver and skeletal muscle confirms these results. The use of a tissue-specific promoter is a first step to restrict transgene expression to a particular cell type of the targeted tissue.

7/7/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09000394 97064956

Heart muscle-specific gene expression using replication defective recombinant adenovirus.

Rothmann T; Katus HA; Hartong R; Perricaudet M; Franz WM
Innere Medizin III, University of Heidelberg, Germany.

Gene Ther (ENGLAND) Oct 1996, 3 (10) p919-26, ISSN 0969-7128
Journal Code: CCE
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Adenoviruses are a promising vector system for future gene therapy of heart muscle diseases. The promiscuous tissue tropism of adenoviruses, however, may lead to the undesirable expression of putative therapeutic genes in nontarget cells and hence to considerable safety limitations for this vector system. To restrict gene expression to cardiomyocytes we constructed an adenoviral vector (Ad-mlcLuc) in which the luciferase gene is under the control of the ventricle-specific myosin light chain -2 (mlc-2v) promoter . For controls, we constructed a recombinant adenovirus without promoter (Ad-Luc) and one with the Rous sarcoma virus (RSV) promoter (Ad-rsvLuc). Our data demonstrate that the newly established viral vector Ad-mlcLuc was specifically active in rat neonatal cardiomyocytes in vitro but not in three established cell lines. Injections of the recombinant adenoviruses into the cardiac cavity of neonatal rats resulted in myocardial specific gene expression of Ad-mlcLuc in vivo, despite the fact that viral DNA was detected by PCR at different levels in all tissues investigated. In vitro and in vivo, Ad-mlcLuc was exclusively active in cardiac muscle cells, reaching 8-9% of the RSV-induced luciferase activity. Direct injection of Ad-mlcLuc into thigh muscle gave only background luciferase activity (0.05% of Ad-rsvLuc). Therefore, in the adenoviral system, the mlc-2v promoter allows heart-specific expression of a foreign gene thus providing a promising tool for gene transfer targeted to the myocardium.

7/7/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08952685 97195688

CARP, a cardiac ankyrin repeat protein, is downstream in the Nkx2-5 homeobox gene pathway.

Zou Y; Evans S; Chen J; Kuo HC; Harvey RP; Chien KR

American Heart Association-Bugher Foundation Center for Molecular Biology, Department of Medicine, University of California, San Diego, La Jolla 92093, USA.

Development (ENGLAND) Feb 1997, 124 (4) p793-804, ISSN 0950-1991 Journal Code: ECW

Languages: ENGLISH

Document type: JOURNAL ARTICLE

To identify the molecular pathways that guide cardiac ventricular chamber specification, maturation and morphogenesis, we have sought to characterize factors that regulate the expression of the ventricular myosin light chain - 2 gene, one of the earliest markers of ventricular regionalization during mammalian cardiogenesis. Previously, our laboratory identified a 28 bp HF-1a/MEF-2 element in the MLC-2v promoter region, which confers cardiac ventricular chamber-specific gene expression during murine cardiogenesis, and showed that the ubiquitous transcription factor YB-1 binds to the HF-1a site in conjunction with a co-factor. In a search for interacting co-factors, a nuclear ankyrin-like repeat protein CARP (cardiac ankyrin repeat protein) was isolated from a rat neonatal heart cDNA library by yeast two-hybrid screening, using YB-1 as the bait. Co-immunoprecipitation and GST-CARP pulldown studies reveal that CARP forms a physical complex with YB-1 in cardiac myocytes and immunostaining shows that endogenous CARP is localized in the cardiac myocyte nucleus. Co-transfection assays indicate that CARP can negatively regulate an HF-1-TK minimal promoter in an HF-1 sequence-dependent manner in cardiac myocytes, and CARP displays a transcriptional inhibitory activity when fused to a GAL4 DNA-binding domain in both cardiac and noncardiac cell context. Northern analysis revealed that carp mRNA is highly enriched in the adult heart, with only trace levels in skeletal muscle. During murine embryogenesis, endogenous carp expression was first clearly detected as early as E8.5 specifically in heart and is regulated temporally and spatially in the myocardium. Nkx2-5, the murine homologue of Drosophila gene tinman was previously shown to be required for heart tube looping morphogenesis and ventricular chamber-specific myosin light chain -2 expression during mammalian heart development. In Nkx2-5(-/-)embryos, carp expression was found to be significantly and selectively reduced as assessed by both whole-mount in situ hybridizations and RNase protection assays, suggesting that carp is downstream of the homeobox gene Nkx2-5 in the cardiac regulatory network. Co-transfection assays using a dominant negative mutant Nkx2-5 construct with CARP promoter -luciferase reporter constructs in cardiac myocytes confirms that Nkx2-5 either directly or indirectly regulates carp at the transcriptional level. Finally, a carp promoter -lacZ transgene, which displays cardiac-specific expression in wild-type and Nkx2-5(+/-) background, was also significantly reduced in

Nkx2-5(-/-) embryos, indicating that Nkx2-5 either directly or indirectly regulates carp promoter activity during in vivo cardiogenesis as well as in cultured cardiac myocytes. Thus, CARP is a YB-1 associated factor and represents the first identified cardiac-restricted downstream regulatory gene in the homeobox gene Nkx2-5 pathway and may serve as a negative regulator of HF-1-dependent pathways for ventricular muscle gene expression.

7/7/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08612735 96222506
The utility of fluorescent in vivo reporter genes in molecular cardiology.
Doevendans PA; Becker KD; An RH; Kass RS
Department of Cardiology, Cardiovascular Research Institute Maastricht, The Netherlands.
Biochem Biophys Res Commun (UNITED STATES) May 15 1996, 222 (2) p352-8, ISSN 0006-291X Journal Code: 9Y8
Languages: ENGLISH
Document type: JOURNAL ARTICLE
In vivo reporter genes can be used in different ways in molecular cardiology. In this paper studies are presented using the green fluorescent protein and one of its mutants, S65T-GFP, as in vivo reporter genes. With this new molecular tool we studied cell type specificity of the murine ventricular myosin light chain 2 promoter , positive cell identification prior to patch clamp procedures, and the use of fluorescence activated cell sorting of transiently transfected mammalian cells.

7/7/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08544787 96157360
Transactivation of cardiac MLC - 2 promoter by MyoD in 10T1/2 fibroblast cells is independent of E-box requirement but depends upon new proteins that recognize MEF-2 site.
Goswami SK; Siddiqui MA
Department of Anatomy and Cell Biology, SUNY Health Science Center at Brooklyn 11203, USA.
Cell Mol Biol Res (UNITED STATES) 1995, 41 (3) p199-205, ISSN 0968-8773 Journal Code: BSK
Languages: ENGLISH
Document type: JOURNAL ARTICLE
MyoD-mediated activation of skeletal muscle genes, which is dependent upon the consensus E-box sequence, involves, at least in one group of muscle genes, another transcription factor, the myocyte enhancer factor-2 (MEF-2). Since the cardiac myosin light chain -2 (MLC -2) gene promoter lacks the functional E-box but contains the activator MEF-2 site, we tested the effect of ectopic expression of MyoD on cardiac MLC -

2 promoter function. Here, we demonstrate that either transient or stable expression of MyoD in otherwise nonpermissive C3H10T1/2 fibroblast cells can promote the expression of MLC - 2 /CAT. Deletion and site-specific mutation analysis demonstrate that the MEF-2 site (Element B) in the MLC - 2 promoter is the target of activation by MyoD. Gel mobility shift assay using nuclear extracts from the normal and MyoD-transfected fibroblast cells did not show a difference in the major MEF-2 binding complexes, except for one complex of fast-moving mobility, which suggested that new MEF-2-like regulatory proteins are induced by MyoD.

7/7/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08543484 96156686
[A transgenic animal model: new possibilities for cardiovascular research]
Transgene Tiermodelle: neue Moglichkeiten fur die Herz-Kreislauf-Forschung.
Franz WM; Frey N; Muller O; Kubler W; Katus HA
Innere Medizin III, Heidelberg.
Z Kardiol (GERMANY) 1995, 84 Suppl 4 p17-32, ISSN 0300-5860
Journal Code: XW7
Languages: GERMAN Summary Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL English Abstract
By means of molecular biology and genetic research the influence of genetic factors on a great variety of human diseases could be shown. In the field of cardiovascular research the genetic defects of a few monogenetic disorders, such as Marfan's syndrome and hypertrophic cardiomyopathy, have been characterized. In addition, candidate genes for polygenetic diseases, such as arterial hypertension and atherosclerosis, have been cloned. However, the identification of a candidate gene or its mutation does not prove its influence on the phenotype or the final cause of a particular disease. Only a targeted manipulation of a defined candidate gene in a transgenic animal model helps to understand the role of the gene and its product in the whole organism. Transgenic experiments can be divided into gene-addition and gene-deletion models. In a gene-addition experiment a fusiongene is microinjected into a fertilized oocyte. The fusiongene itself consists at least of a regulatory element promoter and of a DNA sequence coding for the gene product (protein) of intended overproduction. The choice of the right promoter is important for obtaining tissue-specific gene expression. The cardiac myosin light chain -2 promoter for example leads to a ventricle-specific gene expression in cardiomyocytes from early embryogenesis through adulthood. In a gene-deletion experiment on the other hand, the target gene is selectively knocked out by homologous recombination in embryonic stem (ES) cells. The selected ES-cells are then injected into blastocysts. If the ES-cells are integrated into the germ line and transmitted to the progeny, a transgenic line is established. This review article describes planning and development of transgenic animals and discusses established transgenic animal model

systems with regard to cardiovascular physiology. In addition, animal models which may provide a basis for future gene therapy will be introduced. (105 Refs.)

7/7/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08234424 95074069
Raf-1 kinase activity is necessary and sufficient for gene expression changes but not sufficient for cellular morphology changes associated with cardiac myocyte hypertrophy.
Thorburn J; McMahon M; Thorburn A
Department of Human Genetics, University of Utah, Salt Lake City 84112.
J Biol Chem (UNITED STATES) Dec 2 1994, 269 (48) p30580-6, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Around the time of birth, cardiac muscle cells lose the capacity to divide and, from this time on, growth of the heart occurs by hypertrophy where each cells gets bigger. The hypertrophic response is characterized by changes in gene expression including expression of the atrial natriuretic factor (ANF) and myosin light chain -2 (MLC -2) genes. In cultured neonatal ventricular myocytes, hypertrophy also involves reorganization of contractile proteins into sarcomeric units. We have investigated the role of the Raf-1 kinase in this response. Activation of an estradiol-regulated Raf-1 protein kinase led to activation of mitogen-activated protein (MAP) kinase and activated expression from the ANF and MLC -2 promoters . Raf-1-induced activation of these genes was inhibited by a kinase deficient mutant of the 44-kDa MAP kinase, Erk1 indicating a requirement for MAP kinases in the Raf-1-induced response. However, activation of Raf-1 was not sufficient to induce the organization of actin into sarcomeric units. Transfection of dominant negative Raf-1 inhibited phenylephrine-induced activation of the ANF and MLC -2 promoters . Transactivation was rescued by the introduction of increased amounts of c-Raf suggesting a role for Raf-1 in the response to alpha-adrenergic agonists. These results suggest that activation of Raf-1 kinase is a critical component of the signal transduction pathway leading to changes in gene expression associated with hypertrophy but that Raf-1 is not sufficient for the regulation of actin organization during the hypertrophic response.

7/7/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07859939 94119074
Positive regulatory elements (HF-1a and HF-1b) and a novel negative regulatory element (HF-3) mediate ventricular muscle-specific expression of myosin light - chain 2-luciferase fusion genes in transgenic mice.

Lee KJ; Hickey R; Zhu H; Chien KR

Department of Medicine, University of California, San Diego School of Medicine, La Jolla 92093.

Mol Cell Biol (UNITED STATES) Feb 1994, 14 (2) p1220-9, ISSN 0270-7306 Journal Code: NGY

Contract/Grant No.: HL-36139, HL, NHLBI; HL-45069, HL, NHLBI; HL-46345, HL, NHLBI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The cardiac myosin light-chain 2v (MLC-2v) gene has served as a model system to identify the pathways which restrict the expression of cardiac muscle genes to particular chambers of the heart during cardiogenesis. To identify the critical cis regulatory elements which mediate ventricular chamber-specific expression of the MLC-2v gene in the in vivo context, a series of transgenic mice which harbor mutations in putative MLC -2 cis regulatory elements in a 250-bp MLC -2 -luciferase fusion gene which is expressed in a ventricular chamber-specific fashion in transgenic mice were generated. These studies demonstrate that both components of HF-1 (HF-1a and HF-1b/MEF-2) are required to maintain ventricular chamber-specific expression and function as positive regulatory elements. Mutations in another conserved element (HF-2) are without statistically significant effect on ventricular chamber expression. Transgenics harboring mutations in the E-box site also displayed significant upregulation of reporter activity in the soleus, gastrocnemius, and uterus, with a borderline effect on expression in liver. Mutations in another conserved element (HF-3) result in a marked (> 75-fold) upregulation of the luciferase reporter activity in the soleus muscle of multiple independent or transgenic founders. Since the HF-3 mutations appeared to have only a marginal effect on luciferase reporter activity in liver tissue, HF-3 appears to function as a novel negative regulatory element to primarily suppress expression in muscle tissues. Thus, a combination of positive (HF-1a/HF-1b) and negative (E-box and HF-3) regulatory elements appear to be required to maintain ventricular chamber-specific expression in the in vivo context.

7/7/15 (Item 15 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

07614364 93380108

Heart-specific targeting of firefly luciferase by the myosin light chain- 2 promoter and developmental regulation in transgenic mice.

Franz WM; Breves D; Klingel K; Brem G; Hofschneider PH; Kandolf R

Department of Virus Research, Max-Planck-Institut fur Biochemie, Martinsried, FRG.

Circ Res (UNITED STATES) Oct 1993, 73 (4) p629-38, ISSN 0009-7330 Journal Code: DAJ

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Based on hybridization studies indicating constitutive expression levels of the endogenous myosin light chain - 2 (MLC -2) gene in embryonic, fetal, and adult myocardium, a model system for selective

targeting of genes to the heart of transgenic mice has been developed. A 2.1-kb DNA fragment of the 5' flanking region of the rat cardiac MLC -2 gene was fused to the firefly luciferase reporter gene and introduced into fertilized mouse oocytes. In four independent transgenic mouse lines, the expression of the MLC -2 -luciferase fusion gene was found exclusively in heart muscle. In contrast to the endogenous MLC - 2 gene, no luciferase activity was detectable in slow-twitch skeletal muscle or any other tissue of transgenic mice. This result suggests that the 2.1-kb DNA fragment of the 5' flanking region of the cardiac MLC -2 gene contains the regulatory elements required for selective gene expression in cardiac myocytes in vivo. In contrast to the endogenous steady-state MLC -2 expression during development, transgenic luciferase activity was 10-fold higher during embryogenesis, when formation of the ventricular loop and septum takes place. The enhanced luciferase activity in early heart development may suggest a growth-dependent control mechanism, involving either transcriptional or posttranscriptional regulation. In conclusion, this model system with the 2.1-kb ventricle-specific MLC -2 promoter sequence should facilitate the overexpression of gene products in the developing and mature heart muscle and further elucidate molecular mechanisms of myocardial diseases such as cardiomyopathies.

7/7/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07341924 92348455

Myosin light chain- 2 luciferase transgenic mice reveal distinct regulatory programs for cardiac and skeletal muscle-specific expression of a single contractile protein gene.

Lee KJ; Ross RS; Rockman HA; Harris AN; O'Brien TX; van Bilsen M; Shubeita HE; Kandolf R; Brem G; Price J; et al

Department of Medicine, University of California, San Diego, School of Medicine, La Jolla 92093.

J Biol Chem (UNITED STATES) Aug 5 1992, 267 (22) p15875-85, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: HL-36139, HL, NHLBI; HL-45069, HL, NHLBI; HL-46345, HL, NHLBI; +

Languages: ENGLISH

Document type: JOURNAL ARTICLE

To examine the relationship between the cardiac and skeletal muscle gene programs, the current study employs the regulatory (phosphorylatable) myosin light chain (MLC -2) as a model system. Northern blotting, primer extension, and RNase protection studies documented the high level expression of the cardiac MLC -2 mRNA in both mouse cardiac and slow skeletal muscle (soleus). Transgenic mouse lines harboring a 2100- or a 250-base pair rat cardiac MLC -2 promoter /luciferase fusion gene were generated, demonstrating high levels of luciferase activity in cardiac muscle, and only background luminescence in slow skeletal muscle and non-muscle tissues. As assessed by in situ hybridization, immunofluorescence, and luminescence assays of luciferase reporter activity in various regions of the heart, both the endogenous MLC -2 gene and the MLC - 2 luciferase fusion gene were expressed exclusively in the

ventricular compartment, with expression in the atrium at background levels. Point mutations within the conserved regulatory sites HF-1a and HF-1b significantly cripple ventricular muscle specificity, while mutation of the single E-box site was without effect, suggesting that ventricular muscle-specific expression occurs through an E-box-independent pathway. This study provides direct evidence that the cis regulatory sequences in the cardiac/slow twitch MLC -2 gene which confer cardiac and skeletal muscle-specific expression can be clearly segregated, suggesting that distinct regulatory programs may have evolved to control the tissue-specific expression of this single contractile protein gene in cardiac and skeletal muscle.

7/7/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07159688 92195291
A ubiquitous factor (HF-1a) and a distinct muscle factor (HF-1b/MEF-2) form an E-box-independent pathway for cardiac muscle gene expression.
Navankasattusas S; Zhu H; Garcia AV; Evans SM; Chien KR
Department of Medicine, University of California, San Diego, La Jolla 92093-0613.
Mol Cell Biol (UNITED STATES) Apr 1992, 12 (4) p1469-79, ISSN 0270-7306 Journal Code: NGY
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Recent studies have identified a conserved 28-bp element (HF-1) within the rat cardiac MLC -2 gene which confers cardiac muscle-specific and inducible expression during myocardial cell hypertrophy. Utilizing a combination of independent experimental approaches, this study characterizes two cardiac nuclear factors which bind to HF-1, a ubiquitous factor (HF-1a), and an A + T-rich binding factor (HF-1b) which is preferentially expressed in differentiated cardiac and skeletal muscle cells. The HF-1a binding site is located in a core region of the 28-bp conserved element, immediately upstream from the A + T-rich HF-1b site, which is homologous to the MEF-2 site found in a number of muscle genes. By a number of separate criteria (gel mobility shift, competition, and mutagenesis studies), HF-1b and MEF-2 appear to be indistinguishable and thus are either identical or closely related muscle factors. Transient assays of luciferase reporter genes containing point mutations throughout the 28-bp HF-1 regulatory element document the importance of both the HF-1a and HF-1b sites in transient assays in ventricular muscle cells. In the native 250-bp MLC -2 promoter fragment, mutations in the single E box had little effect on cardiac muscle specificity, while point mutations in either the HF-1a or HF-1b binding site significantly reduced promoter activity, underscoring the importance of both the HF-1a and HF-1b sites in the transcriptional activation of this cardiac muscle gene. Thus, this study provides evidence that a novel, ubiquitous factor (HF-1a) and a muscle factor (HF-1b/MEF-2) can form a novel, E-box-independent pathway for muscle-specific expression in ventricular cardiac muscle cells.

7/7/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07159084 92159038

Transcriptional activation of the cardiac myosin light chain 2 and atrial natriuretic factor genes by protein kinase C in neonatal rat ventricular myocytes.

Shubeita HE; Martinson EA; Van Bilsen M; Chien KR; Brown JH

Department of Pharmacology, University of California at San Diego, School of Medicine, La Jolla 92093.

Proc Natl Acad Sci U S A (UNITED STATES) Feb 15 1992, 89 (4) p1305-9, ISSN 0027-8424 Journal Code: PV3

Contract/Grant No.: HL28143, HL, NHLBI; HL36139, HL, NHLBI; HL45609, HL, NHLBI; +

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A cultured myocardial cell model was used to examine the role of protein kinase C-dependent pathways in the transcriptional activation of two cardiac muscle genes [myosin light chain 2 (MLC -2) and atrial natriuretic factor (ANF)] during alpha-adrenergic receptor-mediated hypertrophy. Phorbol ester (phorbol 12-myristate 13-acetate) and the alpha-adrenergic agonist phenylephrine both activate protein kinase C (PKC) and induce 4- to 5-fold increases in the expression of MLC -2 and ANF promoter /luciferase reporter genes with little effect on Rous sarcoma virus/luciferase or minimal prolactin promoter /luciferase genes. To further assess the role of PKC in cardiac gene regulation, PKC expression vectors encoding constitutively activated PKC-alpha or PKC-beta, or a catalytically inactive PKC, were transiently cotransfected with the cardiac promoter /luciferase constructs. Cotransfection of either activated PKC-alpha or PKC-beta cDNA induces the expression of MLC -2 and ANF promoter /luciferase genes and of a reporter gene responsive to the transcription factor AP-1. The Rous sarcoma virus/luciferase and minimal prolactin promoter /luciferase genes are not concomitantly induced by cotransfectin with the PKC genes, indicating specificity of the transcriptional effect. The finding that activated PKC increases cardiac gene transcription suggests that activation of this enzyme may be a proximal signal for coregulation of two cardiac genes, MLC -2 and ANF, during the course of myocardial cell hypertrophy.

7/7/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07048689 92217021

fos-mediated repression of cardiac myosin light chain- 2 gene transcription.

Goswami SK; Zarraga AM; Martin ME; Morgenstern D; Siddiqui MA

Department of Anatomy and Cell Biology, State University of New York Health Science Center, Brooklyn 11203.

Cell Mol Biol (UNITED STATES) Feb 1992, 38 (1) p49-58, ISSN 0145-5680 Journal Code: CPV

Contract/Grant No.: 1R01 HL43159, HL, NHLBI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The transcription of the chicken cardiac myosin light chain -2 (MLC - 2) promoter containing a 1.3 Kb 5'-flanking DNA segment is repressed upon co-transfection with an expression vector (pMMV) containing the proto-oncogene fos in embryonic chicken cardiac muscle cells in culture. Similar concentrations of co-transfectants containing other genes e.g. luciferase were ineffective. To identify the DNA element(s) in MLC - 2 gene that responds to fos-mediated inhibition, 5'-sequential deletion mutants of MLC - 2 promoter were tested in a transient transfection assay. A mutant, in which the 5' distal sequence was deleted upto -1200 bp upstream of the mRNA start site was sensitive to fos inhibition, but the mutant containing -1130 bp was not, suggesting that a fos responsive element (FRE) is located between -1130 to -1200 bp upstream of the transcription initiation site. The same FRE sequence was also responsive to fos-inhibition in chicken skeletal muscle cells as well. Since over-expression of fos is implicated in repression of myogenic process, the selective inhibition of MLC -2 promoter activity by fos and identification of FRE sequence potentially important in understanding the relationship between myogenesis and the oncoprotein-mediated signal pathway(s).

7/7/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06987843 90337996
Alpha- and beta-adrenergic stimulation induces distinct patterns of immediate early gene expression in neonatal rat myocardial cells. fos/jun expression is associated with sarcomere assembly; Egr-1 induction is primarily an alpha 1-mediated response.
Iwaki K; Sukhatme VP; Shubeita HE; Chien KR
Department of Medicine, University of California, La Jolla 92093.
J Biol Chem (UNITED STATES) Aug 15 1990, 265 (23) p13809-17, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The present study was designed to determine if alpha- and beta-adrenergic stimulation of neonatal rat myocardial cells might induce common and/or distinct members of the immediate early gene program and to assess whether their induction might correlate with the differential effects of these adrenergic agonists on cardiac gene expression, sarcomere assembly, and several features of myocardial cell hypertrophy. Alpha- and beta-adrenergic stimulation of neonatal rat myocardial cells both produce an increase in the assembly of an individual contractile protein (myosin light chain -2) into organized sarcomeric units and also rapidly induce mRNAs for the immediate early genes c-fos and c-jun, thereby suggesting a potential role for these protooncogenes in sarcomerogenesis. alpha-Adrenergic stimulation results in the co-induction of mRNAs encoding a zinc finger protein gene (Egr-1). However, beta-adrenergic stimulation does not produce a significant increase in the levels of Egr-1 mRNA, providing the first

evidence in any cell system that c-fos and Egr-1 expression can regulated separately. Studies with norepinephrine in combination with various adrenergic receptor antagonists revealed that the induction of Egr-1 is primarily an alpha 1-mediated, pertussis toxin-insensitive response. These studies provide the first evidence for the induction of immediate early genes following adrenergic stimulation of myocardial cells and demonstrate alpha- and beta-adrenergic stimulation can rapidly activate the expression of common and distinct subsets of these transcriptional regulators . Since alpha- and beta-adrenergic agonists have differential effects on cardiac gene expression and on the acquisition of several features of myocardial cell hypertrophy, the induction of Egr-1 provides a potential mechanism for the induction of genes that are exclusively induced during alpha-adrenergic-mediated myocardial cell hypertrophy.

7/7/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06942077 91210299

Co-regulation of the atrial natriuretic factor and cardiac myosin light chain- 2 genes during alpha-adrenergic stimulation of neonatal rat ventricular cells. Identification of cis sequences within an embryonic and a constitutive contractile protein gene which mediate inducible expression.

Knowlton KU; Baracchini E; Ross RS; Harris AN; Henderson SA; Evans SM; Glembotski CC; Chien KR

Department of Medicine, University of California, San Diego, La Jolla 92093.

J Biol Chem (UNITED STATES) Apr 25 1991, 266 (12) p7759-68, ISSN 0021-9258 Journal Code: HIV

Contract/Grant No.: HL-36139, HL, NHLBI; HL26-H, HL, NHLBI; NS-25037, NS, NINDS

Languages: ENGLISH

Document type: JOURNAL ARTICLE

To study the mechanisms which mediate the transcriptional activation of cardiac genes during alpha adrenergic stimulation, the present study examined the regulated expression of three cardiac genes, a ventricular embryonic gene (atrial natriuretic factor, ANF), a constitutively expressed contractile protein gene (cardiac MLC -2), and a cardiac sodium channel gene. alpha 1-Adrenergic stimulation activates the expression and release of ANF from neonatal ventricular cells. As assessed by RNase protection analyses, treatment with alpha-adrenergic agonists increases the steady-state levels of ANF mRNA by greater than 15-fold. However, a rat cardiac sodium channel gene mRNA is not induced, indicating that alpha-adrenergic stimulation does not lead to an increase in the expression of all cardiac genes. Studies employing a series of rat ANF luciferase and rat MLC -2 luciferase fusion genes identify 315- and 92-base pair cis regulatory sequences within an embryonic gene (ANF) and a constitutively expressed contractile protein gene (MLC -2), respectively, which mediate alpha-adrenergic-inducible gene expression. Transfection of various ANF luciferase reporters into neonatal rat ventricular cells demonstrated that upstream sequences which mediate tissue-specific expression (-3003 to -638)

can be segregated from those responsible for inducibility. The lack of inducibility of a cardiac Na+ channel gene, and the segregation of ANF gene sequences which mediate cardiac specific from those which mediate inducible expression, provides further insight into the relationship between muscle-specific and inducible expression during cardiac myocyte hypertrophy. Based on these results, a testable model is proposed for the induction of embryonic cardiac genes and constitutively expressed contractile protein genes and the noninducibility of a subset of cardiac genes during alpha-adrenergic stimulation of neonatal rat ventricular cells.

7/7/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06299180 88203190
The promoter of the chicken cardiac myosin light chain 2 gene shows cell-specific expression in transfected primary cultures of chicken muscle.
Arnold HH; Tannich E; Paterson BM
Department of Toxicology, Medical School, University of Hamburg, FRG.
Nucleic Acids Res (ENGLAND) Mar 25 1988, 16 (6) p2411-29, ISSN 0305-1048 Journal Code: O8L
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Transcriptional regulation of the chicken cardiac myosin light chain 2 (MLC2 -A) gene was investigated in chicken primary myoblast and fibroblast cultures transfected with vector constructs containing the bacterial marker gene for chloramphenicol acetyltransferase (CAT) under the control of the MLC2 -A promoter . We here demonstrate that sequences close to the TATA box are sufficient to direct muscle specific and regulated expression of the MLC2 -A mRNA. Transcription from MLC2 -A promoter /CAT hybrids in myocytes starts from the authentic cap site that is also used in vivo. In primary breast muscle cells, bromodeoxyuridine (BUdR), a reversible blocking agent of cell differentiation, suppresses transcription from the MLC2 -A promoter whereas nonmuscle promoters like the RSV- or the cytoplasmic beta-actin promoter are unaffected in their transcriptional capacity. Although the endogenous cardiac MLC2 -A gene in chicken is exclusively active in heart, the transfected MLC 2 -A promoter escapes this cell type control in primary cultures of breast muscle. These results demonstrate that although muscle specificity of the MLC2 -A gene and its transcriptional up-regulation during differentiation is maintained in a rather short promoter segment, restrictive elements determining the muscle cell type specificity in vivo are either not present in our constructs or are not acting under the conditions of transient transfection.

7/7/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06114361 87008629
Characterization of 5'-flanking region of heart myosin light chain 2A gene. Structural and functional evidence for promoter activity.
Zarraga AM; Danishefsky K; Deshpande A; Nicholson D; Mendola C; Siddiqui MA
J Biol Chem (UNITED STATES) Oct 15 1986, 261 (29) p13852-60, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Two recombinant clones, lambda LC5 and lambda LC13, encompassing the entire regulatory myosin light chain 2 (MLC2A) gene of chicken heart muscle were isolated. Of these, lambda LC5 which contains a large 5'-flanking sequence of about 7.0 kb, was characterized by a partial nucleotide sequence analysis. A TATA-like sequence (TATTTTTA) and a CAAT-box (CAAAAGT) are located at positions -32 and -59, respectively, which most likely constitute the functional promoter region in the gene. Based on primer extension reaction with a synthetic 20-mer corresponding to the 5'-leader sequence and total poly(A+) RNA, the probable transcription initiation site in the gene was located. The gene promoter activity was demonstrated following transient expression of recombinant genomes containing the chicken upstream sequence fused to the bacterial chloramphenicol acetyltransferase (CAT) or to the rat preproinsulin II genes. The extracts from a Quail fibroblast cell line (QT35) transfected with the construct (pLCo5.2iCat) containing the putative chicken promoter , and the CAT gene promoted the formation of 3'-acetate chloramphenicol. Another construct (pBC12LC5.2f) contains the rat preproinsulin II gene placed under the control of chicken promoter and a simian virus 40 origin of replication. Transfection of COS cell line with pBC12LC5.2f DNA resulted in an efficient expression of rat preproinsulin mRNA initiating from the chicken promoter . The transfection assay also allowed detection of chicken MLC2A gene transcripts by S1-nuclease protection of end-labeled DNA probes. A comparison of the MLC2A upstream gene sequence with those available for skeletal myosin light chains revealed no common sequence elements, suggesting that cardiac MLC2A gene promoter region has diverged considerably from its counterparts in skeletal muscle.

7/7/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06112981 86310773
Characterization of the myosin light- chain- 2 gene of Drosophila melanogaster.
Parker VP; Falkenthal S; Davidson N
Mol Cell Biol (UNITED STATES) Nov 1985, 5 (11) p3058-68, ISSN 0270-7306 Journal Code: NGY
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Recombinant DNA clones encoding the Drosophila melanogaster homolog of the vertebrate myosin light -chain -2 (MLC -2) gene have been isolated. This single-copy gene maps to the chromosomal locus 99E. The nucleotide sequence was determined for a 3.4-kilobase genomic fragment

containing the gene and for two MLC -2 cDNA clones generated from late pupal mRNA. Comparison of these sequences shows that the gene contains two introns, the positions of which are conserved in the corresponding rat sequence. Extension of a primer homologous to the mRNA reveals two start sites for transcription 12 nucleotides apart. The sequence TATA is not present ahead of the mRNA cap site. There are two major sites of poly(A) addition separated by 356 nucleotides. The protein sequence derived from translation of the cDNA sequence shows a high degree of homology with that for the DTNB myosin light chain (MLC -2) of chicken. A lower degree of sequence homology was seen in comparisons with other evolutionarily related calcium-binding proteins. RNA blots show high levels of expression of several transcripts during the developmental time stages when muscle is being produced. In vitro translation of hybrid-selected RNA produces two polypeptides which comigrate on two-dimensional gels with proteins from Drosophila actomyosin, although the cDNA sequence reveals only one 26-kilodalton primary translation product.

7/7/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05641120 90036891
Structure, organization, and expression of the rat cardiac myosin light chain- 2 gene. Identification of a 250-base pair fragment which confers cardiac-specific expression.

IDS

Henderson SA; Spencer M; Sen A; Kumar C; Siddiqui MA; Chien KR
Department of Medicine, University of California, San Diego, School of Medicine, La Jolla 92093.
J Biol Chem (UNITED STATES) Oct 25 1989, 264 (30) p18142-8, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The present study characterized the structure, organization, and expression of the rat cardiac myosin light chain (MLC) -2 gene. The rat cardiac MLC -2 gene has seven exons which display complete conservation with the exon structure of the rat fast twitch skeletal MLC -2 gene. A 250-base pair (bp) sequence of the 5'-flanking region contains CArG motifs and additional cis elements, each greater than 10 bp in length, which were conserved in sequence and relative position with the chick cardiac MLC -2 gene. A series of MLC -2 /luciferase fusion genes consisting of nested 5' deletions of the MLC - 2 5'-flanking region were constructed and transfected into primary neonatal rat myocardial cells and a non-myocardial cell line (CV-1), demonstrating that this 250 bp of the MLC -2 5'-flanking region was sufficient to confer cardiac specific expression on a luciferase reporter gene. This study suggests the presence of important proximal regulatory sequences in the MLC -2 5'-flanking region which are capable of directing the cardiac specific expression of the rat cardiac myosin light chain 2 gene.

7/7/26 (Item 26 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05466850 89218994
Regulatory myosin light-chain genes of Caenorhabditis elegans.
Cummins C; Anderson P
Department of Genetics, University of Wisconsin, Madison 53706.
Mol Cell Biol (UNITED STATES) Dec 1988, 8 (12) p5339-49, ISSN 0270-7306 Journal Code: NGY
Contract/Grant No.: GM30132, GM, NIGMS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
We have cloned and analyzed the Caenorhabditis elegans regulatory myosin light-chain genes. C. elegans contains two such genes, which we have designated mlc-1 and mlc - 2 . The two genes are separated by 2.6 kilobases and are divergently transcribed . We determined the complete nucleotide sequences of both mlc-1 and mlc -2 . A single, conservative amino acid substitution distinguishes the sequences of the two proteins. The C. elegans proteins are strongly homologous to regulatory myosin light chains of Drosophila melanogaster and vertebrates and weakly homologous to a superfamily of eucaryotic calcium-binding proteins. Both mlc-1 and mlc - 2 encode abundant mRNAs. We mapped the 5' termini of these transcripts by using primer extension sequencing of mRNA templates. mlc-1 mRNAs initiate within conserved hexanucleotides at two different positions, located at -28 and -38 relative to the start of translation. The 5' terminus of mlc - 2 mRNA is not encoded in the 4.8-kilobase genomic region upstream of mlc -2 . Rather, mlc -2 mRNA contains at its 5' end a short, untranslated leader sequence that is identical to the trans-spliced leader sequence of three C. elegans actin genes.

7/7/27 (Item 27 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05047264 87222590
Cloning and characterization of mammalian myosin regulatory light chain (RLC) cDNA: the RLC gene is expressed in smooth, sarcomeric, and nonmuscle tissues.
Taubman MB; Grant JW; Nadal-Ginard B
J Cell Biol (UNITED STATES) Jun 1987, 104 (6) p1505-13, ISSN 0021-9525 Journal Code: HMV
Contract/Grant No.: HL01724, HL, NHLBI; HL07101, HL, NHLBI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The 20-kD regulatory light chain (RLC) plays a central role in the regulation of smooth muscle contraction. Little is known about the structure or expression of smooth muscle myosin light chain (MLC) genes. A cDNA library was constructed in the expression vector , lambda gt-11, with mRNA derived from cultured rat aortic smooth muscle cells. Using antibody generated against tracheal smooth muscle myosin, three cDNA clones encoding a RLC were isolated, one of which, SmRLC-2, represents a full-length transcript of the RLC mRNA. The derived amino acid sequence shows 94.2% homology with the chicken gizzard RLC, and 70 and 52% homology with the rat

skeletal and cardiac muscle MLC -2 proteins, respectively. Thus, the gene encoding the putative smooth muscle RLC appears to have originated by duplication of the same ancestor that gave rise to the sarcomeric MLC -2 genes. Contrary to the stringent tissue-specific expression of sarcomeric MLC - 2 genes, RNA blot hybridization and S1 nuclease mapping demonstrates that the putative smooth muscle RLC gene is expressed in smooth, sarcomeric, and nonmuscle tissues at significant levels. Primer extension analysis suggests that the same promoter region is used in these different tissues. Thus the putative smooth muscle RLC gene appears to be a gene that is constitutively expressed in a large variety of cells and has a differentiated function in smooth muscle.

7/7/28 (Item 1 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

11015505 BIOSIS NO.: 199799636650
In vitro, in vivo and in ovo heart-specific expression using an adenoviral vector.

AUTHOR: Griscelli Frank; Gilardi Pascale; Opolon Paule; Chianale Colette; Perricaudet Michel; Ragot Thierry
AUTHOR ADDRESS: Lab. Genetique des Virus Oncogenes, CNRS URA 1301, Inst. Gustave Roussy, Villejuif, France

JOURNAL: Journal of Molecular and Cellular Cardiology 29 (5):pA39 1997

CONFERENCE/MEETING: XVIII European Section Meeting of the International Society for Heart Research Versailles, France July 2-5, 1997
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LANGUAGE: English

7/7/29 (Item 2 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

10831208 BIOSIS NO.: 199799452353
Early mortality and developmental anomalies in chick embryos examined without and after introduction of reporter LacZ gene.

AUTHOR: Sokol-Misiak Wanda; Rogulska Teresa
AUTHOR ADDRESS: Polish Academy Sci., Inst. Genetics and Animal Breeding, Dep. Molecular Biol., Jastrzebiec, 05-551 M, Poland

JOURNAL: Animal Science Papers and Reports 14 (4):p211-221 1996
ISSN: 0860-4037
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English; Polish

ABSTRACT: Studied was the quality of fertilized eggs of individual hens in basic population (Sx and RIR strains) in order to obtain the hens giving the embryos of best quality which can be used for chick/chick transgenic chimaera construction. Hens which yielded the best embryos (no unfertilized eggs, or dead or abnormal embryos) were used as mothers of next generation (daughters). Significant differences were found (Duncan test) in mortality of embryos obtained from contemporaries and mothers (frequency = 0.099 and 0.000, respectively, P lt 0.01) and no statistically significant differences between embryos obtained from mothers and daughters. However, the malformations were more frequent in daughters' embryos as compared with mothers' (frequency = 0.038 and 0.000, respectively, P lt 0.01). It strongly suggests that only frequency of early embryonic mortality depends on maternal genome. The chick/chick transgenic chimaeras were constructed by injection of Sx transfected blastodermal cells into subgerminal cavity of RIR blastoderms in freshly laid, unincubated egg. The expression vectors (consisted of LacZ gene driven by different promoters) were used for transfection. The abnormal embryos (absent in the population of mothers) were similar in chick/chick transgenic chimaeras and control embryos of mother's contemporaries and mother's daughters. They had deformed heads with often anteriorly open brain. There is no evidence that introduced gene vectors were responsible for abnormalities which were observed in chick/chick chimaeras. In 3 day chick/chick chimaeras, the transcription of introduced gene driven by tissue unspecific SV 40 or RSV promoter was observed in extraembryonic tissues. The LacZ gene (driven by tissue specific promoter - chicken cardiac myosin light chain -2 gene promoter) was not expressed.

7/7/30 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

06369123 EMBASE No: 1996020964
The potential of transgenic animal models in cardiovascular research
TRANSGENE TIERMODELLE: NEUE MOGLICHKEITEN FUR DIE HERZ-KREISLAUF-FORSCHUNG

Franz W.M.; Frey N.; Muller O.; Kubler W.; Katus H.A.
Innere Medizin III, Bergheimer Str. 58,D-69115 Heidelberg Germany
Zeitschrift fur Kardiologie (Z. KARDIOL.) (Germany) 1995, 84/SUPPL. 4 (17-32)

CODEN: ZKRDA ISSN: 0300-5860
DOCUMENT TYPE: Journal; Conference Paper
LANGUAGE: GERMAN SUMMARY LANGUAGE: GERMAN; ENGLISH

By means of molecular biology and genetic research the influence of genetic factors on a great variety of human diseases could be shown. In the field of cardiovascular research the genetic defects of a few monogenetic disorders, such as Marfan's syndrome and hypertrophic cardiomyopathy, have been characterized. In addition, candidate genes for polygenetic diseases, such as arterial hypertension and atherosclerosis, have been cloned. However, the identification of a candidate gene or its mutation does not

prove its influence on the phenotype or the final cause of a particular disease. Only a targeted manipulation of a defined candidate gene in a transgenic animal model helps to understand the role of the gene and its product in the whole organism. Transgenic experiments can be divided into gene-addition and gene-deletion models. In a gene-addition experiment a fusion gene is microinjected into a fertilized oocyte. The fusiongene itself consists at least of a regulatory element promoter and of a DNA sequence coding for the gene product (protein) of intended overproduction. The choice of the right promoter is important for obtaining tissue-specific gene expression. The cardiac myosin light chain -2 promoter for example leads to a ventricle specific gene expression in cardiomyocytes from early embryogenesis through adulthood. In a gene-deletion experiment on the other hand, the target gene is selectively knocked out by homologous recombination in embryonic stem (ES) cells. The selected ES-cells are then injected into blastocysts. If the ES-cells are integrated into the germ line and transmitted to the progeny, a transgenic line is established. This review article describes planning and development of transgenic animals and discusses established transgenic animal model systems with regard to cardiovascular physiology. In addition, animal models which may provide a basis for future gene therapy will be introduced.

7/7/31 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0213061 DBA Accession No.: 97-08182 PATENT
Nucleic acid construct for gene therapy containing promoter of myosin light chain gene- dystrophin, beta-adrenergic receptor or nitric-oxide-synthase gene transfer using an adeno virus, adeno-associated virus or liposome vector, for heart disease gene therapy
AUTHOR: Franz W M; Rothmann T; Katus H A
CORPORATE SOURCE: Gross Groenau, Germany.
PATENT ASSIGNEE: Franz W M 1997
PATENT NUMBER: WO 9717937 PATENT DATE: 970522 WPI ACCESSION NO.: 97-289035 (9726)
PRIORITY APPLIC. NO.: DE 1040630 APPLIC. DATE: 961001
NATIONAL APPLIC. NO.: WO 96DE2181 APPLIC. DATE: 961114
LANGUAGE: German
ABSTRACT: A new DNA construct for use in gene therapy contains a human or rat regulatory sequence (RS) from the 5'-end of the heart myosin light chain gene (MLC -2), functionally attached to a sequence encoding a therapeutic product, antisense RNA or ribozyme. The RS extends from +18 to -19 up to at least -800, preferably -2700, from the MLC -2 gene start site, and includes elements HF-1a, HF-1b, MLE1 and HF-3, and optionally the E-box and/or HF-2, and the CSS element. The DNA may be incorporated in a defective adeno virus or adeno-associated virus vector , with 2 inverted terminal repeats, or a liposome. The therapeutic gene may encode dystrophin, beta-adrenergic receptor or nitric-oxide-synthase (EC-1.14.13.39), with optional non-coding regions and/or a polyadenylation signal. The construct provides high

transfection rates, stable gene expression, and is particularly specific for cardiac muscle. The DNA may be used to treat heart disease, particularly cardiac insufficiency, dilatative or hypertrophic cardiomyopathy, dystrophinopathy, vascular disease, hypertension, arteriosclerosis, stenosis and/or restenosis. (44pp)

7/7/32 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0196795 DBA Accession No.: 96-08175
Heart muscle-specific gene expression using replication-defective recombinant adeno viruses- for heart muscle disease gene therapy (conference abstract)
AUTHOR: Franz W M; Rothmann T; Perricaudet M; Kuebler W; Katus H A
CORPORATE AFFILIATE: Univ.Heidelberg Inst.Gustave-Roussy German-Cancer-Res.Inst.
CORPORATE SOURCE: Innere Medizin III, University of Heidelberg, 69120 Heidelberg, Germany.
JOURNAL: J.Mol.Cell.Cardiol. (28, 5, A36) 1996
ISSN: 0022-2828 CODEN: JMCDAY
CONFERENCE PROCEEDINGS: International Society for Heart Research, XVII European Congress, Bologna, Italy, 18-21 June, 1996.
LANGUAGE: English
ABSTRACT: Adeno viruses are a promising vector system for gene therapy of heart muscle disease. However, the promiscuous tissue tropism of adeno viruses may lead to undesirable expression of putative therapeutic genes in non-target cells, and to considerable safety limitations. To restrict gene expression to cardiomyocytes, the ventricle-specific activity of a myosin light chain -2 (mlc -2) promoter was used to drive expression of a luciferase reporter gene, in Ad-mlcLuc. Controls were constructed with no promoter (Ad-Luc) and with a Rous-sarcoma virus (RSV) promoter (Ad-rsv-Luc). Ad-mlcLuc was specifically active in neonatal cardiomyocytes in vitro. Injection of Ad-mlcLuc viruses (2,000 million pfu) into the chamber of the left ventricle and thigh muscle of neonatal rats confirmed cardiac-specific gene expression in vivo. The mlc - 2 promoter construct was exclusively active in cardiac muscle cells, reaching 8% of the strong RSV promoter activity. This vector is a promising tool for gene transfer targeted to the myocardium. (0 ref)

7/7/33 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0170955 DBA Accession No.: 94-13506
Characterization of a cardiac-specific and developmentally upregulated promoter in transgenic mice- myosin- light- chain- 2 promoter application in transgenic mouse heart tissue-specific gene expression and development-specific gene expression; potential gene therapy (conference abstract)

AUTHOR: Franz W M; Katus H A; Brem G; Kandolf R
CORPORATE AFFILIATE: Max-Planck-Inst.Biochem. Univ.Heidelburg Inst.Mol.Anim.Breed.Munchen
CORPORATE SOURCE: Max-Planck-Institute for Biochemistry, D-8033 Martinsreid, Germany.
JOURNAL: J.Cell.Biochem. (Suppl.18A, 250) 1994
CODEN: JCEBD5
LANGUAGE: English
ABSTRACT: A model system for targeting of genes to the heart of transgenic mice was developed. A 2.1 kb DNA fragment of the 5'-flanking region of the rat cardiac myosin light chain -2 (MLC -2) gene was fused to a firefly luciferase (EC-1.13.12.7) gene and introduced into fertilized mouse oocytes. In 4 independent transgenic mouse lines, the expression of the fusion gene was exclusively found in the heart muscle. In contrast to the endogenous MLC -2 gene, which was constitutively expressed in ventricles and outflow tract during cardiac embryogenesis and coexpressed in slow twitch skeletal muscle in adult mice, 2 properties of the 2.1 kb MLC -2 promoter were observed: (1) a cardiac muscle restricted activity in embryonal, fetal and adult mice with no coexpression in any other tissues; and (2) a 10-fold upregulated expression activity during the embryonal period of ventricular loop and septum formation. The 2.1 kb fragment contained the regulatory elements for selective gene expression in cardiac myocytes in vivo. The promoter may facilitate foreign gene expression in gene therapy. (0 ref)
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